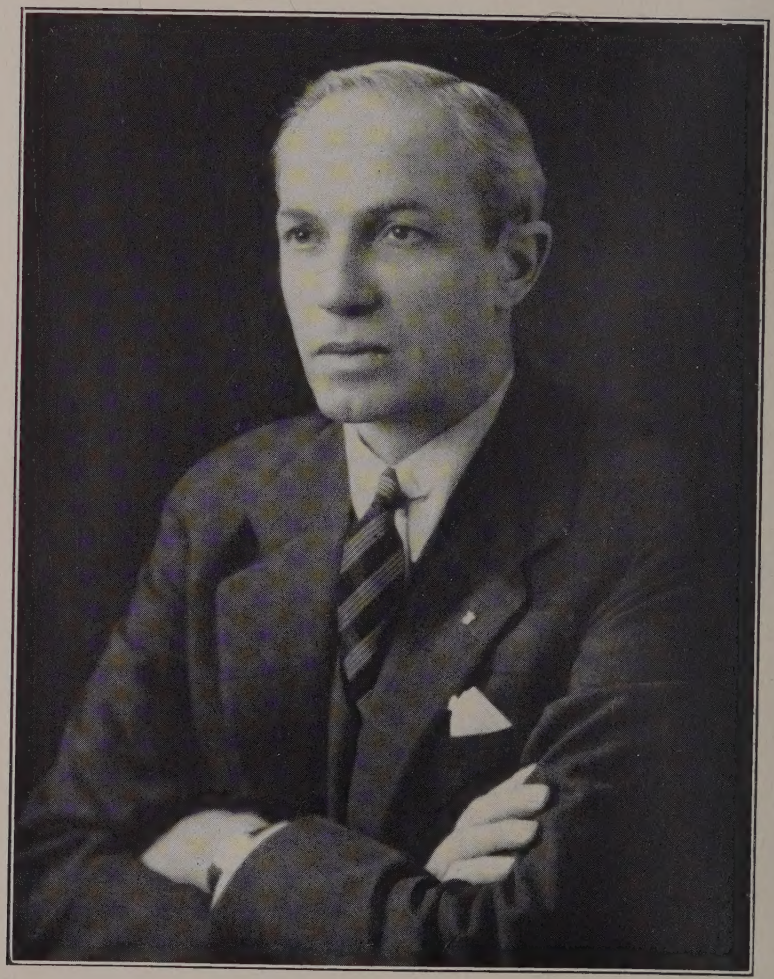

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LIFE HISTORY STUDIES AND THE DEVELOPMENT OF PARASITOLOGY*

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I wish to express to the council and members of the American Society of Parasitologists my deep appreciation of the honor conferred in my election as your president. It was my good fortune to be a charter member of the Society and during one-third of its existence to serve as secretary. For the opportunities to be of service, I thank you in all sincerity.

A precedent has been set, and it appears to me to be a good one, that the retiring president shall discuss some topic of general significance in the field of parasitology, one in which he has a special interest. While I should be among the last to advocate the maintenance of traditions merely because of their antiquity, and indeed our Society is yet a youthful organization, it appears wise to cherish and observe those precepts which have proved their worth and usefulness. Accordingly, since my efforts for the past ten years have been devoted largely to the study of life cycles of animal parasites, it seems pertinent that I should address my remarks to a consideration of this subject. After discussing certain aspects of life history work, I hope to show the part which these studies have had in the development of parasitology.

It would be well perhaps at this point to recall that the accumulation of knowledge progresses inexorably in natural, logical, orderly sequence. Each forward step is dependent upon previous steps, and each in turn prepares the way for further advancement. To paraphrase a line from a poem known to almost every schoolboy of my generation, "We build the ladder by which we rise from . . . (ignorance to understanding), and we mount to its summit round by round." There is no short cut to knowledge and most sudden or chance discoveries are actually the outgrowth of years of patient, preliminary investigation. Legions of previous workers have recorded their observations and experience and, in fact, present knowledge rests upon and consists of such accumulated experience. Further progress is possible only because of past attainment, and its rate depends upon the acuity with which existing knowledge is

*Address of the Retiring President, American Society of Parasitologists, December 29, 1939, Columbus, Ohio.

brought to bear upon unsolved problems. The organization, correlation and reorientation of available facts direct the approach to new discoveries and have led to the great scientific generalizations.

In the general advance of the biological sciences, as the subject became subdivided through more intensive study and specialization, it is apparent that common trends have been followed in each section of the field. Earliest observations pertained to the appearance and habits of plants and animals. More precise and detailed knowledge of morphology led to the delineation of species and their arrangement in taxonomic groups. With information concerning the structure of organ systems, it became possible to investigate the manner in which they develop and function; so studies on gross morphology led to the development of histology, embryology and physiology. Inspection of the more obvious features was followed later by examination of the more minute and concealed structures and the more intricate and subtle processes. The study of morphology and physiology, moreover, led inevitably to the realization that the interrelations of organisms afforded an immense field for investigation,—a sociology of the most extensive kind, one which embraces every living thing.

One of the most widespread ecological relations is that of parasitism. In this discussion, the subject is restricted to a consideration of the relationship among animal species. As old and as universal as biotic associations, the parasitic mode of life has permeated almost every group of animals, where the individuals are themselves parasites or serve as hosts for parasitic forms. The parasites may be parasitized in turn; as for example, the gregarines, cestodes, trematodes and nematodes, which harbor microsporidia; the opalinids, which contain amoebae; and the trematodes, whose excretory system is infected with flagellates. Consideration of the extent and incidence of parasitism indicates more parasitic than free-living species and more parasitic than free-living individuals. Furthermore, it is apparent that parasitism comprises one of the most distinctive categories of animal existence and constitutes a discrete biological phenomenon which shows certain definite and fundamental characteristics in every group of animals which has adopted the habit. The same general tendencies are manifest, similar attributes and relationships appear, and similar consequences inevitably follow the adoption of the parasitic habit. Parasitology may thus be recognized as one of the biological sciences. This fact was clearly demonstrated in the presidential address delivered before this Society by the late Dr. Maurice C. Hall. With a happy combination of subtle humor, pungent wit and hard common sense, he established the scientific status of parasitology. It is my purpose now to discuss the contribution which life history studies have made to its development.

The parasitic species have been derived from free-living progenitors by gradual and progressive adaptation. The parasitic habit has arisen, at

least in most instances, as a result of attempts to secure either protection or food. There are only a few basic conditions of existence, and as Schiller observed with fine discernment, "The edifice of the world is maintained through the impulses of hunger and love," i.e., the instincts of self preservation and reproduction. These innate and impelling motives are primarily responsible for the development of parasitism. Animals in the same habitat strive for sustenance, for the means of subsistence and survival. The stronger prey upon the weaker, but some of the small and physically weak are able for various reasons to impose themselves upon the strong and profit thereby. They may begin as lodgers or temporary parasites but ultimately they remain permanently, and receive both protection and food from the host. Finally, to secure safer and more advantageous conditions of life, the ectoparasite invades the body of the host. Some of the endoparasites may have been accidentally introduced, but whatever the origin, they all come to share the same fate,—modification of form with attendant alterations in metabolism and life cycle.

Among ectoparasitic species the life cycle undergoes little change; it remains direct and relatively simple. Reproduction gives rise to larvae or young individuals which develop into adults like their parents. For perpetuation of the species, it is essential that dispersive stages of the parasite leave the original host and establish the infection of new hosts. The transfer to new hosts is accomplished either by contact or by migration. Ectoparasites of aquatic animals usually have the ability to swim during some stage of the life cycle, and such activity aids in dispersal of the species and in the acquisition of new hosts. Ectoparasites of terrestrial animals are limited to those which can resist desiccation, and hence, such species are largely confined to members of the phylum ARTHROPODA. In their life cycles, successive stages, separated by molts, may occur on different animals, but the course of development is monogenetic and the infection of new hosts by juvenile stages is the rule. Apparently the general opinion that all ectoparasites have direct life cycles, has resulted in a neglect of these forms, and little careful work has been done on the subject. Such investigations are needed and should yield important results.

Endoparasitism is correlated with marked changes in the life cycle, and the complicated life histories usually associated with parasitism are found among the highly specialized protozoan and helminthic species. Invasion of the host can not be successfully consummated without accompanying modification of the life cycle, for the adoption of the endoparasitic habit entails certain difficulties and dangers on the species which assumes it. Once an individual enters a host, usually it remains there until it perishes. If the progeny of such a parasite were also to remain in the original host and continue multiplication, the host itself might be

imperiled, and the destruction of the host would be fatal to the parasite. In order that the parasitic species may be perpetuated, it is essential that the offspring of the parasite should leave the first host and secure new hosts, and incidentally, the infection of new hosts provides for the distribution as well as survival of the species. As a rule, therefore, the endoparasite gives rise by reproduction to individuals which pass out from the host. These are usually cysts in protozoa and either eggs or larvae in helminths. Such dispersive forms are infective stages in the life cycle and provide for dissemination of the parasite. In certain instances they reenter the original host species; in others they attack different animals which serve as intermediate hosts. The dispersive stages of endoparasites are frequently quite unlike their immediate progenitors, and succeeding stages, which live in different environments, may manifest such disparity in structure and activity that their genetic relations would hardly be suspected. The course of the life history, as it has been traced in representatives of various groups, shows much diversity and the modifications appear to be adaptations to different and changing environments, in which survival has been the determining factor.

The life cycles of parasitic protozoa manifest all gradations from the simple to the very complex. Species which infect open cavities usually produce cysts which remain dormant or show limited division, but which await chance introduction into a suitable host. They are not greatly modified as a result of parasitism, and since sexual reproduction is rare or absent, the life cycle is monogenetic. The protozoa which live in blood or other tissues are often transferred from one animal to the next by an intermediate host which acts as a vector or carrier and in which reproduction may occur. In sporozoan species alternation of sexual with asexual reproduction prevails, but there are never more than two hosts in the life cycle. The SPOROZOA are a heterogeneous collection of amoeboid and flagellate forms which have become cell or tissue parasites and in which metagenesis has been superimposed on multiple fission. Most of them were primitively parasites of invertebrates, while a few have secondarily acquired additional invertebrate or vertebrate hosts and a digenetic life cycle. When alternation of hosts occurs, the asexual multiplicative phases take place in one host and the sexual phases in the other.

Among the cestodes, except for the monozoic forms which may be secondarily simplified in form and life history, both asexual and sexual reproduction occur in the definitive host, and multiplication in larval stages is rare. The eggs contain microscopic six-hooked embryos, encased in resistant coverings. In the great majority of cases they are ingested by arthropods, which serve as the first intermediate hosts. So far as present information extends, this relation obtains in all members of the TETRAPHYLLIDEA, DIPHYLLIDEA, TETRARHYNCHIDEA and PSEUDOPHYLLIDEA. Most of these cestodes are parasites of fishes, while the

remainder use fishes as second intermediate hosts and mature in fish-eating vertebrates. Members of the TETRAPHYLLIDEA appear ordinarily to have two intermediate hosts in the life cycle, but it is probable that the second intermediate or transfer host is not essential. Present knowledge concerning the diphyllidean and tetrarhynchid cestodes is too incomplete for the formulation of a general statement. Members of the PSEUDOPHYLLIDEA infect all classes of vertebrates, and one genus, *Archigetes,* contains species, possibly neotenic, which may attain sexual maturity in the coelom of oligochaete annelids. Most of the pseudophyllidean cestodes have a three host life cycle, in which the first intermediate host is a crustacean. Fishes serve generally as second intermediate hosts, and definitive hosts other than fishes probably acquired the parasites in a piscine diet. In the aberrant family CARYOPHYLLAEIDAE, if it is correctly included in the PSEUDOPHYLLIDEA, the life cycle is reduced; annelids serve as intermediate hosts, and further studies are required to determine the status and affinities of these unusual forms. The presence of larval stages in annelids does not necessarily imply however that the caryophyllaeids are distinct from other pseudophyllideans, since I have found fork-tailed larvae of a marine trematode in the coelom of the annelid *Hydroides.* Although the adult stage of the fluke is unknown, it is almost certainly a member of one of the recognized families.

Cyclophyllidean cestodes are predominantly parasites of birds and mammals, with a few species in reptiles and amphibians. The life cycle of these cestodes typically involves two larval stages and a single intermediate host. The first infective larvae, the onchospheres, develop while the eggs are still in the proglottid. Such embryonated eggs pass from the intestine of the primary host, and with rare exceptions, develop further only after ingestion by a suitable intermediate host in which the onchospheres emerge and penetrate the intestinal wall. If the intermediate host is an invertebrate, the onchosphere invades the body cavity and completes the second larval stage, developing into a cysticercoid larva which contains the scolex of the future cestode with its adhesive organs, ready for attachment when introduced into the intestine of the primary host. Cysticercoid larvae have been found in crustaceans, insects, myriapods, mites, oligochaetes, leeches and molluscs. If the intermediate host is a vertebrate, the ingested onchosphere traverses the intestinal wall to reach the coelom or else the lymph spaces and veins by which it is transported to the liver. The onchospheres may continue their development in the liver or they may pass to other organs. These larvae often produce fluid-filled vesicles and are then known as cysticerci or bladder worms. In a few species, *Echinococcus* and *Multiceps,* asexual multiplication may occur in the cyst. In any event, the second larval stage is completed when the cysticercoid or cysticercus becomes infective for the final host. When the cysticercoid or cysticercus is ingested, larval coverings if pres-

ent are digested; and if conditions are suitable, the larva attaches itself to the intestinal wall and develops into the sexually mature cestode. In rare instances, viz., species of *Hymenolepis* and *Cylindrotaenia,* development may be completed without the intervention of an intermediate host. The onchospheres may emerge in the intestine of the primary host, penetrate the intestinal wall, and there complete the second larval stage, after which they return to the lumen of the intestine. It is probable that these abbreviated life cycles have resulted from the elimination of a former intermediate host, and in certain species the cycle may be completed either with such a host or by the shorter method.

The rule stated previously, that ectoparasitic forms are monogenetic, whereas endoparasitic ones are digenetic, is well exemplified by the trematodes. The ectoparasitic species constitute the MONOGENEA and the endoparasitic ones the DIGENEA. The apparent exceptions are probably more ostensible than real. The type of reproduction in *Gyrodactylus,* an ectoparasite, is yet uncertain. The endoparasitic polystomes from the urinary bladder of amphibians, representatives of the MONOGENEA—whose near relatives are ectoparasites, appear to have acquired a digenetic life cycle. On the other hand, the aspidogastrids are endoparasitic and monogenetic, although they may have alternation of hosts. Notwithstanding these exceptional instances, review of available data indicates strongly that the MONOGENEA and DIGENEA are distinct in morphology and life history and that they are only distantly related, if, indeed, they have not arisen from different stocks.

Thousands of digenetic trematodes are known by their sexually mature stages, and in recent years the larval stages have received increased attention. In spite of many and repeated investigations, the nature of larval reproduction has not been finally determined. It has been described as metagenesis, heterogeny, extended metamorphosis, paedogenesis, germinal lineage with or without polyembryony, and polymorphism. Grobben (1882) first suggested that a type of heterogeny, the alternation of bisexual reproduction with parthenogenesis, occurs in the DIGENEA, and he expressed the belief that cercariae are produced by the parthenogenetic development of ova. This view was accepted by Sinitsin, who proposed the terms: "marita" to designate the sexually mature stage, and "parthenita" to designate the sporocyst and redial generations. This terminology has been adopted by certain more recent authors and extensively used in the literature. So far as I am aware, parthenogenesis has never been established by critical and competent methods in either sporocysts or rediae, and the best recent work has shown that in several species it does not occur. Accordingly, the terminology of Sinitsin, with its implied connotation, is not only unjustified, but may prove very misleading.

The study of life cycles of digenetic trematodes has been a long and

tedious struggle. Bojanus (1818) observed the emergence of cercariae from rediae, an observation that elicited from Oken the editorial comment, ". . . man möchte nun wetten, dass diese Cercarien Embryonen von Distomen seyen, nur wollen die Augen wieder nicht passen." Mehlis (1831) reported the emergence of ciliated larvae from the eggs of *Monostomum flavum,* and von Siebold (1835) observed rediae within such ciliated larvae. Steenstrup (1842) advanced the idea of metagenesis, and applied it to the reproductive processes of coelenterates, trematodes and other lower organisms. Notwithstanding these significant observations, the first life history was not completed until Leuckart and Thomas, independently in 1882, discovered the cycle in *Fasciola hepatica.* The ensuing forty years witnessed the discovery of only about twenty additional life histories, while the past ten years has been a fruitful period, in which the developmental cycles of scores of species, belonging to most of the families, have been traced.

Among nematodes, the life cycles manifest much variation. The sexes are separate and the females either oviparous or viviparous. The eggs of these worms have been used in the classical cytological studies on maturation, fertilization and cleavage. The larvae may be free-living and the alternation of free-living with parasitic generations is not infrequent. The larvae undergo repeated molts and often a significant change of form. It was an epoch-making discovery when Looss demonstrated the tissue-migrating habit of the hookworm. A masterly review of the migration of larval nematodes and its biological significance was presented by Fülleborn (1929) in an address before the London School of Tropical Medicine.

Although preceded by earlier investigations, the adoption of the experimental method, in the study of the life histories of animal parasites, dates from the period of 1850. It was in that year that Herbst transmitted *Trichinella spiralis* by feeding methods, but Virchow (1858) was the first to rear and recognize sexually mature specimens which he recovered from the intestine of the dog. In 1852 Küchenmeister, after noting morphological similarity of the larvae and adults, demonstrated the life cycle of *Taenia pisiformis,* and the following year P. J. van Beneden discovered the life history of *Taenia solium.* These results were followed shortly by the studies of Leuckart on the development of *Trichinella* and other species of *Taenia.* Leuckart also showed that the nematode *Cucullanus elegans* developed in *Cyclops* and, following his suggestion, Fedtschenko (1871) worked out the life cycle of the Guinea worm. Manson (1877) observed the larvae of *Filaria* in *Culex fatigans* and in 1900 Bancroft and Low completed the cycle. The larva of *Diphyllobothrium latum* was recognized in fishes in 1882, but the cycle was not resolved until 1917 by von Janicki and Rosen. Grassi and Rovelli (1892) found larvae of *Hymenolepis* in the rat flea. Smith and Kilborne (1893) in

their studies on Texas fever, were the first to provide experimental proof that an arthropod could transmit a protozoan disease, and Ross (1898) proved that malaria is transmitted by mosquitos. With the advent of the so-called modern period, i.e., since 1900, application of the experimental method has yielded discoveries, familiar to all and too numerous to mention.

In the discovery of the many life cycles, various methods have been employed, all of which may be resolved into the application of certain basic and fundamental principles. The studies often began with simple, incidental observations which led to more intensive investigation. Naturally, the procedure differed with the organism under consideration, but it should be emphasized that, in completing the life cycle, it is essential for the parasite to use readily available channels of transit. In many cases present life cycles are not primitive, but have been developed as adaptive modifications in accord with changing ecological conditions. When all factors are known, it is clear that the parasite has used the most natural, logical and easiest method to complete its life cycle. Therefore in life history studies, the first steps involve the acquisition of precise and detailed knowledge concerning the morphology of the infective stages of the parasite. The nature of investing coverings, if present, suggest the length of time the stage may remain dormant and the probability of spontaneous emergence. The presence or absence of locomotor structures, of organs for penetration, or of encystment, are significant and afford instructive clews concerning the course of the developmental cycle. The activity and behavior of the larvae and their responses to gravity, light, temperature, contact, or other factors, may be helpful in directing the investigation. Finally, the general character and specific features of the environment in which the infective stages are found, may indicate the next host. So, proper procedure follows the usual scientific method: accurate and intelligent observation, penetrating analysis, and logical deduction. In the conclusion of my first paper on the life history of the anoplocephaline cestodes, after demonstrating the necessity of an intermediate host, I made the prediction, "The problem can be solved and intelligent effort, patience and accurate observation will certainly disclose the intermediate host." That prediction was fulfilled three years later. The idea, prevalent at one time that successful life history studies required some sort of divination, occult power or sixth sense, has vanished along with other superstititons. It has been demonstrated repeatedly that the solution of life history problems involves the same orderly scientific methods as the solution of any other problem which requires experimental proof.

In life history studies, hypotheses developed from analysis and deduction must be tested by controlled experimentation. Such procedure necessitates the infection of experimental animals with appropriate stages of

the parasite. The studies may begin with any stage in the life cycle. Frequently the structure and behavior of the parasite, and the bionomics of the locality in which it occurs, will suggest the next step in the cycle. An essential preliminary condition is the assurance that the animals used for experiments are free from a previous infection which could be confused with the experimental one. The dispersive stages of the parasite are the infective agents, and an abundance of material is advantageous. Large numbers are not necessary, however, as may be illustrated by one instance in my own experience. The trematode, *Typhlocoelum cymbium,* so far as I am aware, has been reported only twice from North America. In the autumn of 1933, I found a single specimen in the nasal cavity of a pied-billed grebe, *Podilymbus podiceps,* killed near New York. The worm was removed to physiological salt solution and soon shed a large number of eggs. It was then fixed, stained and mounted. The eggs were placed in tap water and soon began to hatch. Different species of laboratory-raised snails were put in the dish with the miracidia and infection resulted. The life history was completed from a single *T. cymbium,* the only specimen I have ever seen. The results are admittedly exceptional, but they demonstrate the possibilities of the method employed.

In addition to the well-established method of tracing life cycles by experimental infection of susceptible hosts, a supplementary method gives promise of great usefulness. Studies in progress in my laboratory have demonstrated that the adult and larval stages of several species are identical serologically, and a precipitin technique has been developed which is so sensitive that adult and larval stages of a single species may be identified by its use.

Evidence afforded by the life cycles of closely related species indicates strongly that members of natural systematic groups have a similar course of development, and that within families the likeness is remarkable. Since the life history of at least one representative from each of the larger groups has been traced, the discovery of additional cycles becomes a much easier task. Accordingly, we may expect the solution of life histories in increasing numbers, in all groups of animal parasites.

Although past successes provide valuable clews on the course of development in particular groups, and indicate likely methods of procedure, the investigation of life history problems is beset with peculiar difficulties and possibilities of error. Many existing accounts, dealing with life cycles, are incomplete and fragmentary. Indeed, some of the long accepted work has recently been found to contain important omissions. For example, the discovery of the unpigmented stages in the malarial parasites, which constitute a hitherto unknown phase of the life cycle, shows that the sporozoites do not regularly invade the red blood cells. The infection may be established and persist for some time in the form of unpigmented

parasites in the reticulo-endothelial cells before the erythrocytes are invaded and clinical symptoms appear. The persistence of unpigmented forms in the deeper tissues, for long periods and even after the disappearance of parasites from the peripheral blood, may explain certain types of relapses that have so perplexed malariologists. The instance cited is only one of several, and it demonstrates that until all possible variations and modifications of a life history are explored, conclusions should be regarded as tentative.

One of the most puzzling problems arises from the fact that it is often difficult to complete well-known life histories under laboratory conditions. My first life history attempts were made with blood flukes of turtles. Eggs are abundant in mucous masses passed by the hosts, and miracidia are readily obtained. A single successful experiment, in which miracidia of *Vasotrema* penetrated laboratory-raised specimens of *Physa* and gave rise to naturally emerging cercariae, was reported at the XIth International Congress of Zoology. But despite repeated attempts with the same material, it was impossible to repeat the results. There can be no doubt that success or failure of laboratory experiments depends on delicately adjusted physiological factors, and further information on this subject is sorely needed.

The physiology of animal parasites is very imperfectly known, and the study has encountered serious obstacles. Actually, the physiological processes and metabolic requirements can not be investigated by usual methods because the organisms can not be subjected to controlled environmental conditions without injury or death. On the other hand, their maintenance *in vitro* for long periods has been difficult because of the lack of knowledge concerning these physiological processes and nutritive requirements. This problem should prove amenable to application of the increased knowledge of nutrition in free-living species, and to improvements in technique. At this time I am confident enough to predict that the time is not far distant when worms as well as bacteria and protozoa will be widely cultured outside the bodies of their hosts. Indeed, I have been able to rear the trematode, *Plagitura parva,* from the metacercarial stage to sexual maturity in bacteria-free solutions. The worms were freed of bacteria by repeated washing in sterile salt solutions. The culture medium consisted of the salt-dextrose-Hottinger broth solution previously employed in attempts to grow *Cryptocotyle,* somewhat altered in concentration and seeded lightly with live yeast. Apparently the addition of yeast provided a factor or factors necessary for growth and development. In the absence of yeast no development occurred, and yeast extracts were not efficacious.

In the development of parasitology, life history studies have made three noteworthy contributions. They have aided in the construction of a broader and sounder base for taxonomic and phylogenetic determina-

tion, they have provided the opportunity for studies on physiology and for determination of the factors concerned in susceptibility and resistance to parasitic infections, and finally, they have been and continue to be the essential prerequisite for intelligent and effective control and regulatory measures.

If taxonomy is to have meaning, it must express genetic and phylogenetic relations. In the establishment of such a system, the principle of homogeneity of groups should receive first consideration. The well-known tendency for animals of diverse ancestry to assume a superficial resemblance and converge toward a common morphological type, after living for a long period in the same environment, is especially prevalent among parasitic forms. Thus, among species which have been greatly modified by parasitic adaptation, the morphological criteria applied to free-living animals lose much of their significance. Under such conditions, knowledge of the life cycle and of the larval stages acquires increased importance. Systems based on adult characters alone and others based on larval structures have led to many well-known errors, and information supplied by life history studies has been of great value in arriving at correct systematic determinations.

Difficulties in specific determination, both of adults and of larvae, have resulted in much confusion. Until the extent of natural variation in a species is determined, and its limits defined, final specific determination is impossible. In establishing true genetic relations, the precipitin technique, mentioned earlier in this discussion, gives a measure of relationship and provides a chemical test of specificity. It is conceivable that such chemical tests may prove sensitive enough to provide an entirely new concept of specificity. In any event it is fair to predict, that in certain genera where a large number of similar species have been described, life history studies will reduce many of these specific names to synonymy.

Evidence from many complementary sources leads to the conclusion that parasitic flatworms have existed since early geologic periods, although they have undergone extensive changes in form and life history. In certain species the original hosts have become extinct, and in others, the hosts, serving as food for different animals, have carried their parasites into new locations where they have become established. The parallel evolution of hosts and parasites, with the interpolation of new hosts and the dropping out of former ones, has been discussed in previous papers. The evolution of the cestodes is indicated by life cycles in that group. It is practically certain that they originated as turbellarian parasites of crustaceans, and later became parasites of fishes when the crustaceans were eaten. Most of them retain this type of life cycle. In the fishes, strobilate stages arose by asexual duplication of parts, a method of reproduction not uncommon among flatworms. Moreover, duplication of reproductive organs in the proglottids of *Diplogonoporus, Dipylidium, Moniezia* and

other genera, increases the reproductive capacity and compensates for lack of reproduction in larval stages. Obviously the increase in reproductive activity enhances the probability of survival, and consequently such species have been more successful in the struggle for existence. In only a few coënogenetic types, e.g., *Echinococcus* and *Multiceps,* has asexual reproduction been introduced into the larval stage. When fishes already parasitized by tapeworms were eaten by higher vertebrates, these tapeworms were distributed accordingly. As terrestrial vertebrates evolved, other arthropods replaced the crustaceans and later, in certain instances, the fish as well as the arthropod intermediate hosts have been dropped out of the life cycle and replaced by vertebrate intermediate hosts. The facts afforded by known life cycles are significant, since they demonstrate how the cycles may be modified; i.e., how additional hosts are added to, or are dropped out of, the life cycle. They afford an explanation of the course by which vertebrates have come to replace invertebrates as intermediate hosts of cyclophyllidean tapeworms.

If the principles utilized in tracing the phylogeny of the cestodes are applied to the data afforded by a study of the digenetic trematodes, it is apparent that the history of the CESTODA is paralleled closely by that of the DIGENEA. Both have a turbellarian ancestry and probably originated at about the same time. It appears that molluscs were the first hosts of the digenetic trematodes and that fishes were the first vertebrate hosts. This association may antedate the appearance of the higher vertebrates. The molluscs have remained the first hosts, and as the flukes invaded the terrestrial vertebrates, various methods were adopted to complete the cycle. Cercariae which reach the definitive host passively with its food encyst either in the first host, on vegetation, or in the bodies of various aquatic animals, while those that live in the vascular system penetrate directly into the bodies of their final hosts. The blood flukes of fishes, reptiles, birds and mammals comprise a common group and their evolution was discussed in a former paper. The species which utilize second intermediate or transfer hosts, are able to prolong the life of their cercariae by encystment as metacercariae, and thus increase the probability of completing the cycle. Such a feature is obviously of value for survival, and frequently a number of different animals may serve as transfer hosts. Certain species have added a third intermediate host in the life history, but four-host cycles are relatively few.

The discovery of the unpigmented stages of the malarial parasites has cleared up certain questions concerning the phylogeny of these organisms. Reichenow has shown that there are species which form a consecutive and continuous series from the gregarine parasites of the intestinal epithelium of invertebrates to the malarial organisms which live in the erythrocytes of primates. Accordingly, the telosporidian sporozoans

make up a homogeneous group, in which the successive stages of adaptation to parasitism are clearly manifest.

The foregoing illustrations have been selected to show the value of life history studies in tracing genetic relations in groups of the most highly modified helminthic and protozoan parasites. Since taxonomy merely portrays these relations in graphic form, the life cycle is of fundamental importance in establishing a natural classification of parasitic organisms.

The changes which have occurred in the life cycles and metabolism of parasitic species have been correlated with modifications in their physiological requirements as they became adapted to new conditions and hosts. The problem is complicated by the fact that the life cycles of most parasites comprise two or more successive generations, in which different host species may be infected, and for which, accordingly, the essential conditions and nutritional requirements may be very dissimilar. The alternation of hosts has resulted from adaptive modifications of the life cycle, introduced accidentally and continued because they were of value for dispersal and survival. The factors which determine the physiological relations between parasite and host are, however, quite unknown. The basis of such specificity is chemical and the adjustment has developed gradually during a long period of association. When newer techniques permit the cultivation of parasites outside the bodies of their hosts, it may be possible to perform experiments which will disclose the physiological factors determining host-parasite relations, and find out something really concrete on this subject.

The investigation of susceptibility and resistance to infection by animal parasites is progressing rapidly, and papers on this subject are being presented in increasing numbers before the annual meetings of our Society. At the International Congress for Microbiology held last September, two programs were devoted to recent advances in this field. It is not my purpose at this time to discuss the subject or enter into the controversy regarding the extent to which immunity is local or general, cellular or humoral, or the source and nature of circulating antibodies; and indeed I could do little more than repeat opinions already published. It is sufficient to state that immunity is developed against animal parasites, that the degree is influenced by diet and other factors, and that the reactions have much in common with those which result from bacterial infections.

The extent, prevalence and effects of parasitism have been discussed by many authors. It has long been realized that much of the morbidity and mortality of man and other animals is produced by the activities of animal parasites. The mention of hookworm disease, schistosomiasis, yellow fever, typhus, malaria, sleeping sickness and amebic dysentery suggests at once that the great scourges which have decimated populations are of parasitic origin. It is largely the prevalence of parasitic dis-

eases which has prevented the exploitation of the tropics, the richest and most productive agricultural areas of the world. In addition to the losses incurred through illness and death of man and other animals, those inflicted on crop products are enormous. These data combine to make parasitology a subject of increasing practical importance throughout the world.

An attempt to distinguish between human and veterinary parasitology is unwise, since in both, the principles and methods are identical. Indeed, most human parasites can be transmitted to other animals, and have been acquired from them. Much of our information concerning human parasites has resulted from study of the same or related species in non-human hosts. Advances in the control of yellow fever were held back for twenty-five years, until a susceptible host was found in the rhesus monkey. These facts show the danger, noted by Miss Cram and other workers, of introducing parasites of unknown pathological significance and unknown life histories, by the importation of animals for exhibition or laboratory purposes.

The most acute practical problems are those of parasite control. Maurice C. Hall once proposed an examination for students of veterinary parasitology, which would provide them with sample life histories of all sorts of parasites and ask them to select in the life cycle the weak link at which one might drive, and propose a method of driving at it. He stated, "This is precisely the end to which all work of the Zoological Division aims and the formulation of control measures ultimately falls to the lot of all its staff." Obviously, to devise the most effective methods of control, it is essential that full information concerning the life cycle be available, and the extent to which parasite control has been developed in all parts of the world bears witness to the influence which life history studies have had in the advancement of parasitology.

The development of parasitology began with morphological studies and description of species. Preliminary systems of classification, based on the morphology of adult and of larval stages proved inadequate until the discovery of life cycles made it possible to correlate the successive stages and gave meaning to otherwise detached observations. Further life history studies will facilitate the erection of a natural system of classification of parasitic groups. While morphological studies are fundamental and will continue to form the basis for all others, the development of parasitology now demands information from physiological studies. Some of the difficulties have been sketched and auspicious points for attack have been indicated. Real progress, in this aspect of the science, awaits refinements in technique of *in vitro* culture and serological reactions. These advances in technical procedure are contingent on previous information concerning life cycles, and will provide, in turn, new

weapons for uncovering additional information on the bionomics of the parasites. Thus, the vicious cycle of parasitism is being counteracted by the benign cycle of parasite control. More complete knowledge of life cycles will permit more intelligent and more effective regulatory methods, the reduction of morbidity, the advancement of health, and the conservation of natural resources. The foundations of parasitology have been well laid, and its future development will be accelerated by the introduction of new experimental methods. We may look forward with confidence to the achievements which the future will certainly provide.

THE FOOD OF THE FOWL NEMATODE, *ASCARIDIA LINEATA* (SCHNEIDER)[1]

J. E. Ackert, J. H. Whitlock and A. E. Freeman, Jr.

As an approach to a study of the resistance of chickens to the nematode, *Ascaridia lineata* (Schneider),[2] Ackert and Whitlock (1935) began a series of tests on the nature of the food of the nematodes. The authors wished to compare the viability and growth of *A. lineata* in normally fed chickens with those of *A. lineata* in chickens nourished only by water per os and by parenteral injections of some nutrient solution. Several solutions were tried. One of them, a glucose solution, gave results which made such comparisons possible. After various comparative tests had been made in 1935 on 141 chickens, the studies were continued in 1936 by Ackert and Freeman. The results of both series of experiments are here recorded.

MATERIALS AND METHODS

The chickens used in these experiments were White Leghorns secured as day old chicks. They were raised under conditions slightly modified from those found by Herrick, Ackert and Danheim (1923), to be adequate for chickens raised in confinement. The normal ration for the chickens consisted of corn meal (40 gm), cracked wheat (24 gm), ground oats (14 gm), meat scraps (10 gm), milk powder (5 gm), alfalfa leaf meal (5 gm), and cod liver oil (1 gm). This ration was altered slightly from time to time to correspond with that used at the Kansas State College Poultry Farm. In order to retain adequate vitamins, fresh mixes of the ingredients were made every second week. The ration was hopper-fed to the chickens.

Nutrient solutions used in the parenteral injections included the following: (1) A suspension of egg yolk prepared aseptically in the ratio of one or two egg yolks to 100 cc of sterile Locke's solution, and administered hypodermically; (2) subcutaneous injections of normal chicken serum; and (3) sterile solutions of glucose injected intramuscularly. The glucose was injected in dosages of 1 or 2 gm of glucose per kilogram of body weight. The dosage was approximate rather than accurate. Chickens weighing from 125 to 225 gm were treated as weighing 200 gm, those weighing from 225 to 325 gm were treated as weighing 300 gm, etc. The dosage was gauged by the weight of the chicken at the time it was taken off feed. In preliminary tests, the glucose was admin-

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[1] Contribution No. 206 from the Department of Zoology, Agricultural Experiment Station, Kansas State College of Agriculture and Applied Science, Manhattan, Kansas.

[2] Same as *Ascaridia galli*.

istered per os in 25 per cent solutions, intravenously in 50 per cent solutions (wing veins), intramuscularly in 25 per cent solutions, and subcutaneously in 10 per cent solutions. Of these methods, the most effective in the winter was the intramuscular injections, thrice daily, into the breast muscles of 25 per cent glucose in Locke's solution in the dosage of 2 gm per kilogram of body weight; in the summer, a dosage of 1 gm worked very satisfactorily.

This solution was compounded from a modification of Locke's physiological salt solution and chemically pure dextrose. The solution consisted of 9.00 gm NaCl, 0.42 gm KCl, 0.24 gm $CaCl_2$ and 0.10 to 0.30 gm $NaHCO_3$ dissolved in one liter of sterile distilled water. To 75 cc of this solution, 25 gm of glucose were added to form the injection fluid.

To facilitate injecting, the breast feathers were clipped, the chicken suspended by the feet and the breast sponged with 60 per cent alcohol. A Luer-Lok syringe and 26 gauge needle sterilized in a 10 per cent formalin solution in 70 per cent alcohol was washed with sterile water and filled with the requisite dose of glucose solution from a rubber-stoppered serum bottle. The solution was then injected, 0.10 to 0.08 cc at each insertion, into the pectoral muscles. Occasionally a chicken developed a subcutaneous edema in the pectoral region. These were relieved by small drainage incisions which quickly healed.

The parasite used in these experiments was the large round-worm, *Ascaridia lineata* (Schneider), of chickens which inhabits the anterior portion of the small intestine. It is of very common occurrence in chickens in temperate climates. Ackert (1930) found it in 49 per cent of one thousand farm chickens selected at random near Manhattan, Kans. In chickens less than two months of age, these worms, if present in large numbers, are very detrimental to the hosts. The effects include loss of blood, retarded growth, reduced blood sugar, increased urates, shrunken thymus glands and increased mortality.

The nematode eggs used for parasitizing the chickens were secured from adult worms of approximately the same length. In culturing, the uteri were isolated and placed in a sterile Petri dish. Those portions of the uteri which had fertile eggs were placed in other sterile Petri dishes and the eggs pressed out and covered with sterile distilled water for culture. Six or eight drops of two per cent formalin were added to prevent mold and fungous growth. The best results were obtained by incubating the eggs at temperatures varying from 25° to 30° C for a period of four to five weeks. Such cultures remained viable for an additional month or so.

When the chickens were to be parasitized, the infective eggs (100 ± 10, or 50 ± 5 eggs) were counted in a drop of water on a standard microscopic slide with the aid of a low power compound microscope and mechanical stage. The dose of eggs was wiped off the slide with a piece

of filter paper and placed in the chicken's gullet. After this the chickens were returned to the pens on normal rations until they were divided into the two lots, (1) injected or experimentals, and (2) the controls.

Upon the death of each experimental bird a control was killed and the intestines of the two flushed out by the hydraulic method of Ackert and Nolf (1929). The worms were then isolated, counted, and measured. The length of each worm was determined by having its shadow, magnified six times, thrown on the ground glass of a photographic bellows. The length of the shadow was traced on onion skin paper and the length of the worm ascertained by the aid of a milled tracing wheel. This reduced considerably the percentage of error in measuring.

EXPERIMENTAL DATA

The initial tests were upon five groups of chicks ranging from 5 to 28 days of age. The chicks were parasitized with the same number of embryonated eggs of *A. lineata,* and soon afterward divided into two lots: (1) The experimentals, to be nourished only by water per os and parenteral injections of a nutritive solution, and (2) the controls to receive their regular ration. When the injected chicken died, its control mate was killed and the worms from both collected and compared. By the end of eight days all of the experimental chicks had died and the corresponding controls sacrificed. In this time there was little difference in the worms from the comparative lots, but the results indicated that injections of an egg yolk suspension were more effective in prolonging the lives of the chickens than were those of normal chicken serum.

Concerning the efficacy of the injected solutions, the chickens receiving the yolk suspension lived a maximum of eight days after the parenteral injections were begun, whereas the chickens completely starved except for water, and those injected with normal chicken serum, lived a maximum of four days.

EXPERIMENT I

While the egg yolk suspension prolonged the life of the chickens eight days, the absorption of the yolk was too slow to afford suitable experimental comparisons. Glucose injections were then tried. Twenty-two chicks, three weeks of age, were parasitized with 100 ± 10 eggs of the nematode, *Ascaridia lineata.* Each week, a new set of experimental and control chickens was placed on experiment until 44 chickens had been utilized. In group 1, the experimental chickens received intramuscularly 1 gm of glucose per kilogram of body weight twice a day. One experimental chicken survived five days. No data were obtained from either lot.

The experimental chickens of group 2 were given the same treatment as those of group 1. By the end of six days the experimental chickens

TABLE 1.—*Comparison of the fowl nematodes* (Ascaridia lineata) *from injected chickens with those from the chickens fed normally (controls), Exper. I*

Group number (chickens)	Experimental (injected) chickens					Control chickens			
	Number chickens	Age worms at beginning of injections	Age worms at chicken's death	Average number worms	Average length worms	Number chickens	Age worms at chicken's death	Average number worms	Average length worms
		(*days*)	(*days*)		(*mm*)		(*days*)		(*mm*)
1	4	3–5	0	0	0	4	3–5	0	0
2	3	7	13	10	4	3	13	12	5
3	2	15	25	*	3	2	25	2	12
4	1	15	26	0	0	1	26	3	28
5	1	15	33	0	0	1	33	0	0
6	1	15	34	0	0	1	36	0	0
7	1	21	26	0	0	1	26	4	38
8	1	21	27	0	0	1	27	6	30
9	3	21	28	*	20	3	28	4	41
10	3	28	35	1	28	3	35	6	52
11	1	28	37	0	0	1	37	0	0
12	1	28	38	1	41	1	38	7	46

* Less than one worm.

of this group had died. An average of 10 *A. lineata* was isolated from these chickens as compared with an average of 12 worms from the controls. The worms from the injected chickens averaged 4 mm in length as compared with 5 mm for the worms from the control chickens (Table 1). In group 3, the two injected chickens lived ten days. There was little difference in the number of worms from them and their controls, but the one from an injected chicken was 3 mm in length, while the worms from the controls averaged 12 mm. From groups 4, 5, 6, 7, and 8 no worms were taken from the experimental chickens; whereas, from the controls, worms were isolated from the birds in three of the five tests. On injecting thrice daily 2 gm of glucose per kilogram of body weight, the injected chickens of groups 5 and 6 lived 18 and 19 days, respectively.

By the time the chickens of group 9 were placed in the injecting pens, they had been parasitized 21 days. This allowed time for the worms to grow to a good size before the chickens were subjected to the experimental conditions. The three chickens in this group were killed after 7 days. Only one worm was isolated from the three whereas an average of 4 worms was taken from the three controls. The worm from the experimentals was 20 mm long as compared with an average worm length of 41 mm from the controls. Similar results were obtained from group 10. But one worm was isolated from each of three experimental chickens while an average of 6 worms came from the controls. The length was 28 mm for the worm from the injected chicken as compared with an average worm length of 52 mm from the controls.

In this experiment, it became evident that the injection thrice daily

TABLE 2.—*Comparative numbers and lengths of* Ascaridia lineata *from chickens injected with glucose for seven days, from control chickens fed normally, and from semi-control chickens fed irregularly. All chickens compared were of the same age and breed. Data from Exper. II*

Group* number (chickens)	Age worms	Experimental (injected) chickens		Control chickens		Semi-control chickens	
		Number worms	Average length worms	Number worms	Average length worms	Number worms	Average length worms
	(*days*)		(*mm*)		(*mm*)		(*mm*)
1	10	6	3	5	3	1	3
2	11	0	..	3	4	13	4
3	12	1	4	4	4	0	..
4	13	0	..	0	..	2	4
5	15	3	3	6	4	3	5
6	16	1	5	1	11	0	..
7	17	8	3	0	..	6	3
8	18	7	2	4	11	1	9
9	19	4	6	15	13	2	19
10	20	0	..	3	15	2	8
11	22	1	4	?	?	0	..
12	23	0	..	4	9	2	21
13	24	0	..	6	26	0	..
14	25	1	4	0	..	0	..
15	26	0	..	2	27	0	..

* One chicken per lot except in groups 1 and 9 in which there were 2 chickens in the experimental, the control and the semi-control lots.
? Worms lost.

of 2 gm of glucose solution into the breast muscles of young chickens would keep them alive long enough (18 or 19 days) to make comparisons between the viability and growth of the *A. lineata* in the intestines of chickens deprived of food by mouth and those of the worms of the same age from the control chickens which were fed normally. The results obtained in Experiment I showed that the worms from the injected chickens were somewhat fewer and smaller than were those from the controls.

EXPERIMENT II

While the authors were securing more data on the food of the *A. lineata,* they wished to study the rate of elimination of *A. lineata* from the intestines of the chickens, as Ackert and Herrick (1928) found that the loss of *A. lineata* from a host increased markedly after the 18th day of parasitism. The results of Exper. I showed that such a drop occurred in the numbers of parasites in the control chickens some time between the 13th and 18th day. However, the elimination of parasites was even more marked in the experimental chickens.

The chickens in Exper. II were handled in the following manner: Starting 3 days after they had received 100 ± 10 embryonated eggs of *A. lineata,* each day an experimental chicken was taken off feed and fed parenterally with the standard amount of glucose.[3] Seven days later, unless it had died in the meantime the chicken was killed and the average number and length of worms obtained compared with those from control chickens, which had been parasitized with the same number of eggs at the same time, and had been kept on full feed until sacrificed. Part of the results of Exper. I indicated that irregularly fed chickens lost more worms than did those kept on full feed. To ascertain if irregular feeding is a factor in the incidence of *A. lineata* a third set of chickens called semi-controls was included in Exper. II. From the third day after parasitizing, each day a semi-control chicken was placed on experiment and deprived of food for at least 8 hours of each of the next 7 days. At the end of that time it was sacrificed along with the experimental and control chickens, and the worms from the three compared. The results of Exper. II are given in Table 2.

Beginning with Group I when the worms in the chickens were 10 days old and the chickens had been in the experiment 7 days, 6 worms were taken from the injected chickens, 5 worms from the controls and 1 worm from the semi-controls. The average lengths of the worms were the same, namely, 3 mm. In Group 2, the semi-control chicken had more *A. lineata* than did the controls, but their size averaged about the same. No worms were recovered from the injected chickens.

[3] Injections were made thrice daily (every 8 hours) of 25 per cent glucose in Locke's solution at the rate of 2 gm of glucose per kilogram of body weight of the chicken.

The results from Group 3 afforded comparisons between the worms from the injected and from the control chickens, the former having 1 worm, the latter 4 worms, with only a slight difference in their average size. Comparisons of 15-day *A. lineata* from the three lots were available in Group 5. Six worms were taken from the control and 3 worms from each of the others. Those from the injected birds were smallest, averaging 3 mm long as compared with 4 and 5 mm, respectively, from the other lots (Table 2). Of the worms from the experimental and control chickens in Group 6, those in the controls grew markedly better, averaging 11 mm in length to 5 mm for the *A. lineata* from the injected chickens.

In Groups 8 and 9 in which comparisons of worms were available from all three lots, the numbers of *A. lineata* fluctuated irregularly, but those from the injected chickens were decidedly shorter, 2 mm as compared with 11 mm from the controls and 9 mm from the semi-controls in Group 8; and 6 mm as compared with 13 mm from the controls and 19 mm from the semi-control chickens in Group 9. After 19 days of parasitism fewer worms appeared to be left in the fowls. The 20-day group (No. 10) yielded not a worm from the injected bird, 3 from the control, and 2 worms from the semi-control. Those from the control chickens average 15 mm as compared with an average of 8 mm for the semi-controls. From Group 11, only one worm was available. It came from the injected chicken and showed no growth over the 12-day worms, each worm having been 4 mm in length.

On the 23rd day of parasitism, no worms were available from the injected chickens, while 4 were recovered from the control and 2 from the semi-control lot. The average lengths were 9 mm and 21 mm, respectively. By the 24th day, worms were available only from the control chickens, there having been 6 *A. lineata* averaging 26 mm in length. From the last two groups of chickens parasitized 25 and 26 days, respectively, one worm 4 mm long was taken from the injected chicken (Group 14) and two worms averaging 27 mm from the control chick of Group 15.

While much variation occurred in the results of Exper. II, certain trends appeared to be significant. For example, the worms from the injected chickens never increased more than 3 mm in length, whereas those from the controls made almost a continuous increase of 24 mm in the 16 days of the experiment; and the *A. lineata* from the semi-controls made a somewhat regular increase in length up to 18 mm during the period. The results thus indicate that the young nematodes (*A. lineata*) during 16 days made scarcely any growth in fowl intestines deprived of the usual food taken by mouth; but that in the presence of abundant fowl food, the worms made almost continuous growth during the period, and, that in the semi-controls where food was available only two-thirds of

the time, the growth rate was rather similar to that of the worms from the controls, but not quite equal to it.

As to numbers of worms that remained in the chickens during the first 19 days of the experiment, no marked differences occurred in the 3 lots of chickens under comparison. Since all chickens were given the same number of infective worm eggs, it appears that the semi-control bird of group 2 was unusually susceptible since it had 13 *A. lineata* which was much the largest number from any chicken of the experiment (Table 2). The restricted amount of food given to the semi-control chickens may have been a factor in reducing slightly the number of worms from the chickens of this lot, but the difference was not marked. Worms were of quite common occurrence until the 20th day of parasitism when more negatives began to appear, especially in the injected and semi-control lots. In the controls that were fed normally, the number of worms also fell off somewhat after the 19th day of parasitism. These results tend to confirm the observation of Ackert and Herrick who found that the rate of elimination of the *A. lineata* from the chickens tended to increase after about the 18th or 19th day of parasitism. This was attributed to the greater difficulty of the worms maintaining their positions in the fowl intestine after they had withdrawn their anterior ends from the intestinal mucosa. The injected and semi-control lots of chickens exhibited this phenomenon quite markedly after the 19th day of the present tests, while the controls showed it but little.

EXPERIMENT III

Further experimentation indicated that better comparisons of *A. lineata* would be available from cases in which the young worms became well established in the host chickens before the injections were begun, and also in hosts young enough to permit fairly rapid growth of the worms. Accordingly for Exper. III, which marked the beginning of the second year of the studies, chickens one month of age, after being weighed and matched, were parasitized with 50 ± 5 embryonated eggs of the nematode and returned to the regular pens for a period of seven days.

On the eighth day, half of the chickens selected, (the experimental lot) were placed in confinement pens where they received only water per os and intramuscular injections, every 8 hours, of 25 per cent glucose in Locke's solution at the rate of 1 gm of glucose for each kilogram of body weight. The other half of the chickens, which constituted the control lot, were allowed to range free in the pens on normal rations. Upon the death of each experimental bird its matched control mate was killed and the intestines of the two flushed out in the usual way. The worms were then isolated, counted, and measured as in the previous experiments. As confinement pens with the necessary individual heat units were available

TABLE 3.—*Comparisons of the numbers and lengths of* Ascaridia lineata *from glucose-injected chickens and normally fed chickens. At the age of one month all chickens were given 50 eggs of the nematode. Data from Exper. III*

Age worms	Experimental (injected) chickens				Control chickens			
	Number chickens examined	Total number worms	Average number worms	Average length worms	Number chickens examined	Total number worms	Average number worms	Average length worms
(*days*)				(*mm*)				(*mm*)
8	1	0	..	..	1	2	2.0	3.6
12	1	7	7.0	3.4	1	2	2.0	5.2
13	1	25	25.0	4.5	1	18	18.0	7.7
14	1	0	..	..	1	26	26.0	10.5
15	5	28	5.6	5.3	5	31	6.2	9.8
16	6	19	3.1	3.4	6	80	13.3	13.2
17	8	25	3.1	4.6	8	23	2.8	14.1
18	9	49	5.4	4.4	9	94	10.4	19.6
19	5	25	5.0	4.5	5	12	2.4	21.4
20	2	11	5.5	4.1	2	3	1.5	17.5
21	1	0	..	..	1	5	5.0	20.4
22	6	11	1.8	4.1	6	51	8.5	28.7
23	1	4	4.0	5.7	1	8	8.0	26.8
27	1	1	1.0	3.1	1	2	2.0	40.1
	48	205	4.2	4.4	48	357	7.4	17.4

for only 12 chickens at one time, a test was run upon 12 experimentals and 12 controls. After this another test was run and so forth. The tests were continued until a total of 96 chickens had been used in the experiment. As the conditions of the tests were uniform, the data were combined. They are presented in Table 3.

Late in the evening of the 8th day one of the experimental chickens died. Its control was killed and the intestines of both flushed. Upon examination the experimental bird had no worms, but the control had two worms which averaged 3.6 mm in length. A second experimental chicken died on the 12th day. It had seven worms and its control, two worms. The former averaged 3.4 mm in length and the latter, 5.2 mm. On the 13th day another experimental bird died. Its control was killed and both birds examined. The experimental chicken had 25 worms and the control, 18. The average length of the worms from the experimental individual was 4.5 mm and that of those from the control, 7.7 mm.

The results from the 14th day birds yielded worms only from a control which had 26 *A. lineata* averaging 10.5 mm in length. Five experimental chickens died on the 15th day of the experiment. The 28 worms from them gave an average of 5.6 worms per bird as compared with a 6.2 average from the controls. In sizes the worms from the experimental groups averaged 5.3 mm in length and the controls, 9.8 mm. This difference was an indication that the worms in the normally fed chickens were really making better growth than those in the chickens not fed by mouth. On the 16th day six injected chickens succumbed and, as usual, their controls were at once sacrified. A 3.1 worm average resulted from the experimental chickens and an average of 13.3 worms came from the controls. The former averaged 3.4 mm in length and the latter, 13.2 mm.

Rather similar results were obtained from the groups of chickens examined on the 17th, 18th, 19th, and 20th days of the experiment, during each of which several hosts were examined; all were infected, and the comparisons of the worms gave results much like those from the earlier days of the experiment (Table 3). In this period the average numbers and sizes of worms from the injected chickens remained about the same as those from the 15th and 16th days. In the controls the numbers of worms continued to fluctuate but their size showed an almost continuous increase. This gave further evidence that the worms in the injected chickens were unable to feed effectively from the intestinal epithelium.

On the 21st day worms were available only from the control which had five *A. lineata* that averaged 20.4 mm in length. Six experimental chickens died on the 22nd day of the experiment; 11 worms from them gave an average of 1.8 worms per bird as compared with an 8.5 average from the controls. The worms from the former group had grown only

an average of 4.1 mm, while those from the normally fed birds had increased to an average of 28.7 mm. Very similar results were obtained from the 23rd day of the experiment (Table 3). On the last day, the 27th, one worm from the injected chicken was 3.1 mm long as compared with an average worm length of 40.1 mm for the two worms from the control chickens.

The results from this experiment and from those of the two previous experiments were so conclusive that further experiments of this kind appeared to be unnecessary. During the period of injections, the *A. lineata* in an intestinal lumen containing only water taken per os, the intestinal secretions and a few bacteria, made little growth; whereas the worms in the normally fed chickens grew almost continuously, as is shown

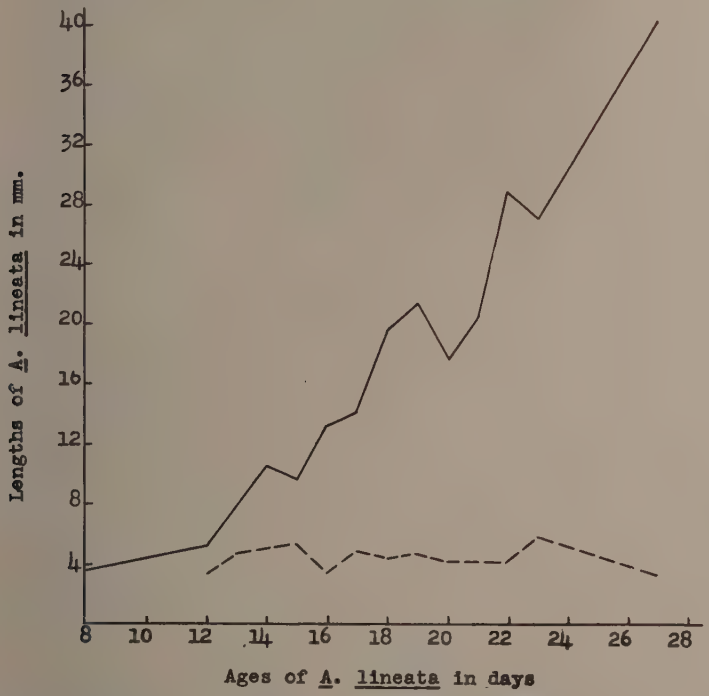

FIG. 1. Comparison of lengths of *A. lineata* from glucose-injected (– – – –) and normally fed (——) chickens.

in Fig. 1. Here it may be seen that the worms from the injected chickens grew slightly until the 15th day of parasitism. After that time there was a reduction in the lengths of the worms with one slight exception on the 23rd day. From the flatness of the curve it would appear that the worms attained most of their growth prior to the eighth day, but exhausted any

reserve material for growth before the 15th day. The fact that the worms obtained on the 23rd day were longer than any others from the experimental chickens might have been due to their being in an especially susceptible individual. The worms in the control chickens, on the other hand, followed closely the growth curve of normal *A. lineata* as demonstrated by Ackert (1931). Thus, the evidence points strongly to the conclusion that the host ingesta forms the principal part of the food of *A. lineata*.

THE BILE

In all of the experiments post mortem examination revealed marked contrasts between the sizes of the gall bladders of the experimental chickens and those of the control chickens. While the gall bladders of the controls were of normal size, those of the glucose-injected chickens were greatly distended. Moreover, in the latter birds, there appeared to be a slow, but almost continuous, flow of bile as shown by the greenish tinged evacuations. As this relatively unchanged bile might serve as a favorable, or as an unfavorable factor for the *A. lineata* in the injected chickens, the bile was experimentally diverted from the portion of the small intestine that the worms inhabit. This was done by making a surgical anastomosis of the duodenum to the ileum and ligating the duodenum posterior to the bile ducts to force the bile through the anastomosis.

After the chickens had been parasitized the usual seven days, the anastomosis was made and the usual injections given. On the 14th day the chicken with the anastomosis died. Seven worms were isolated from this bird and measured. The worms averaged 3.8 mm in length. As no data on *A. lineata* from the regularly injected chickens were available on the 14th day a comparison was made with the averages of the worms recovered from the glucose-injected birds examined on the 13th and 15th days. The anastomosed chicken had somewhat fewer worms than the average for the 13th day, but slightly more than the number obtained on the 15th day. In length, the worms were well within the limits of variation of those of the glucose-injected group (Table 3). From these comparisons it was inferred that the bile had no marked effect upon these worms. No other intestinal secretions were studied.

DISCUSSION

Little study has been made of the nutritive needs of the nematode *Ascaridia lineata* (Schneider), but some information is available on the ASCARIDAE, to which, according to Eisenbrandt (1936), *A. lineata* shows close relationship. The general physiology of the ASCARIDAE was reviewed by Winterstein in 1911 and more recently by McCoy (1935). The nutritive needs of the ASCARIDAE were studied by Flury (1912) who concluded that those of the ASCARIDAE were almost the same as the nutri-

tive needs of other animals, since the qualitative body analysis was approximately the same. Archer and Peterson (1930) and Li (1933 a and b) in studies of the feeding habits of the ASCARIDAE found that the nematodes were stimulated to feed by the presence of solid particles in the host ingesta, but that in the absence of these particles the worms did not feed. These observations appear to be confirmed by the present experiments. In Fig. 1 the growth curve of the *A. lineata* from the glucose-injected chickens shows that in the absence of host ingesta the worms did not grow, especially after the 15th day. Their growth prior to that time may be explained by the work of Giovannola (1936). This investigator, in an analysis of the energy and food reserves of nematodes, found that during the first larval stage a food reserve was built up that was sufficient to carry the larva through a period of lessened nutrition. A similar situation prevailed to some extent in Expers. I, II, and III, but especially in Exper. IV in which the newly hatched larvae of *A. lineata* had normal food during the first seven days of the experiment. This afforded opportunity for the storage of reserve food from which to draw for some time. The reserve appeared to become exhausted in the *A. lineata* about the 15th day as no consistent growth in length of the worms from the injected birds was observed after that time (Fig. 1).

The growth of the *A. lineata* in the injected birds from the seventh to the 15th day might be attributed to tissue feeding, as the larvae are partially imbedded in the intestinal mucosa (Ackert, 1923). But if this were a factor the young *A. lineata* should have continued growing through the 18th day when the last of the larvae supposedly withdrew their anterior ends from the mucosa and lived free in the intestinal lumen. Furthermore, Ackert (1938), who introduced live *A. lineata* into the body cavities of chickens where tissue was available, found the worms unable to thrive. While this failure might have been due in part to the inflammatory condition induced, it, nevertheless, appears that the fowl nematode *A. lineata* is neither able to feed upon tissue nor to obtain its nourishment from the intestinal mucosa as was suggested by Garin (1913) for the ASCARIDAE.

The absence of food in the intestinal tract of the host, however, might cause several factors to become detrimental to the parasites. Chief among these are: Alterations in the pH of the worm habitat, alterations in oxygen supply, secretion of intestinal products poisonous to the worm, alterations in peristaltic actions, alterations of the bacterial flora of the intestinal tract, and lack of food for the parasite.

Concerning the pH, hydrogen-ion determinations were made on the worm habitat of the intestines of freshly killed chickens in Exper. II. The pH range was 6.6 to 7.4 in the experimental chickens, 6.5 to 7.3 in the semi-controls and 6.4 to 7.1 in the control birds. No marked differences among the groups were observed. The range of fluctuation was

within the limits recorded by Ackert (1931) who, for 56 chickens of various ages, found a pH range of 5.7 to 7.5. As the pH range of the experimental chickens in the present experiments was well within the limits of these normally fed chickens, it appears that the reduced numbers and lengths of the *A. lineata* in the experimental chickens were not due to alterations in the hydrogen-ion concentration.

Likewise it is doubtful if alteration of the oxygen supply was a factor. Campbell (1931, 1932) showed that the oxygen tension in the intestine approaches that of other tissues except at the time that the active reduction processes of digestion are in progress. No digestion was taking place in the empty gut of the experimental animals; so there should have been plenty of oxygen available for the parasites if they lead, as is highly probable, an aerobic existence. If they lead an anaerobic existence, as some workers suspect, the presence of the oxygen should not be a detriment to them since *A. lineata* will grow in culture in the presence of oxygen (Ackert, Todd and Tanner, 1938).

As to poisonous substances, Bahrs (1931) concluded that the intestine of a starved rabbit produced a toxic product which influenced the growth-promoting power for planarian worms fed upon the rabbit intestine. If such a factor were present in the starved mucosa of the experimental chickens, it would be expected that the numbers of *A. lineata* would be markedly reduced during the 10th to the 18th day of parasitism when the worms have their anterior ends buried in the mucosa (Ackert and Herrick, 1928). Surely, these larvae so intimately associated with the mucosa would be more susceptible to a poisonous product in it than would the older worms living free in the lumen of the intestine. But the evidence from the present experiments shows that the drop in incidence of parasitism did not occur until after the worms had left the mucosa. It therefore appears that the fewer and shorter *A. lineata* from the experimental chickens were not due to toxic substances secreted by the empty intestine.

Concerning alterations in peristalsis, Ackert and Nolf (1931) obtained results which indicated that lowered peristalsis rate in vitamin B deficient chickens was conducive to the retention of larger numbers of intestinal nematodes (*A. lineata*). Thus, from the standpoint of peristalsis, the empty intestine would favor rather than hinder the retention of the *A. lineata.* On the other hand, the unfed chickens took much water. Sometimes their crops were full of it. The introduction into the intestine of a large volume of water might cause some vermifugal action by increasing peristalsis and removing some worms. Whether the possible vermifugal action would overcome the slowed peristalsis resulting from the starved intestine is problematical. At any rate, the altered peristalsis would not explain the smaller size of the worms in the experimental animals.

Another factor which might become detrimental from the empty digestive tract is an alteration of the bacterial flora of the host intestine. Li (1933a), however, has shown that bacteria are probably unimportant as a food source for the closely related ASCARIDAE since microörganisms were found in the enteric tracts of only half the parasites examined and then in extremely limited numbers. It is conceivable that the slowed peristaltic action of the host intestine due to starvation might result in fewer bacteria than normal being eliminated. Whatever favorable influences this factor might have for the increase of the bacteria population were undoubtedly limited by other influences such as the absence of food for the bacteria, since in no case were there any indications of excessive bacterial growth such as significant alterations of the intestinal pH, manifestations of excessive fermentation in the gut, or symptoms of autointoxication evidenced by the experimental chickens.

The smaller numbers of *A. lineata* in the injected chickens appears to have been due to starvation of the parasites, although the large volumes of water taken at times may have had a slight vermifugal effect. In view of the conditions as discussed, the markedly smaller sizes of the *A. lineata* in the injected chickens than those from the normally fed fowls apparently were due to partial starvation from lack of host ingesta. The nearly empty intestine while furnishing mucus (Ackert, Edgar and Frick, 1939), some bacteria and digestive enzymes failed to provide adequate food for the nematodes.

SUMMARY

1. In studies upon the food of the fowl nematode, *Ascaridia lineata* (Schneider) comparisons were made upon the numbers and lengths of *A. lineata* from chickens nourished only by water per os and intramuscular glucose injections, with the worms from chickens of the same age that were fed normally. Injections of 25 per cent glucose in Locke's solution at eight-hour intervals were the most successful in prolonging the lives of the injected fowls.

2. The results from 237 chickens showed that the worms thrived markedly better in normally fed chickens than in those nourished only by the glucose injections. In fact, the *A. lineata* from the controls made normal growth while those from the glucose-injected chickens made little growth after normal feeding of the fowl ceased.

3. After considering various factors which might arise in the small intestines of the injected chickens and which might become detrimental to the worms, the writers are of the opinion that the fewer and shorter nematodes in the experimental chickens were due to partial starvation.

4. The results of the experiments indicate that the food of the fowl nematode *A. lineata* consists mostly of host ingesta.

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SOME TAXONOMIC CHARACTERS OF CUTEREBRINE (DIPTERA) LARVAE, WITH LARVAL DESCRIPTIONS OF TWO SPECIES FROM GEORGIA

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The species of *Cuterebra* form an interesting group of specialized animal parasites whose life history, habits, and effect on the host are but slightly known. They are for the most part parasites of rabbits, squirrels, and other rodents. Occasionally they invade such domestic animals as the cat and the dog, and even hogs, cattle, or mules.

Lindquist[1] has shown that in the vicinity of Uvalde, Tex., the cotton-tail rabbit acts as an important wild-host reservoir for the primary screwworm, *Cochliomyia americana* C. & P. The warble *Cuterebra buccata* (F.) was found to be the principal predisposing cause for screwworm infestations in cotton-tail rabbits. It is therefore possible that rabbit warbles act as an important predisposing cause for screwworm cases in rabbits in the Southeast.

The purpose of this paper is to point out some taxonomic characters that appear to be of specific value in differentiating cuterebrine larvae and to describe the larvae of two species of *Cuterebra* collected in Georgia, viz., *Cuterebra buccata* from the cotton-tail rabbit, *Sylvilagus floridanus mallurus* Thomas, and *Cuterebra cuniculi* (Clk.) from the marsh rabbit, *Sylvilagus palustris palustris* Bachman.[2] Notes on the life history and habits of these two species are also included.

MATERIALS AND METHODS

The larvae of *Cuterebra buccata* and *C. cuniculi* were obtained from rabbits collected near Valdosta, Ga. The rabbits were either caught alive in a box-type rabbit trap or were brought in alive or dead by hunters. Both live and dead rabbits were examined carefully for living larvae, encysted dead larvae, and old cysts from which mature larvae had dropped. Live infested rabbits were kept alive until the larvae had matured and dropped. These larvae, after careful comparison with preserved material to determine relationship, were placed on moistened sand in quart or pint fruit jars. After the larvae had pupated, the jars in which they lay were placed in a cool closet.

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[1] Lindquist, Arthur W. 1937 Myiasis in wild animals in southwestern Texas. J. Econ. Entom. 30: 735–740.

[2] Determinations of adult *Cuterebra* reared in connection with these studies were made by D. G. Hall, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

All dead rabbits were skinned so that the skin and underlying muscle tissue could be carefully examined. In this way a number of encysted, partly disintegrated specimens, and many cast skins, were found. Furthermore, the small, early instars were more surely located by such an examination.

For a study of the more generalized characteristics of cuterebrine larvae, at least six undetermined species collected in the United States from various hosts, in addition to the two species described, were examined.

NOTES ON LIFE HISTORY AND HABITS

Cuterebra buccata (F.)

The larvae of *Cuterebra buccata* were found in the cotton-tail rabbit during June and November, although various laymen have reported that the warble is found in rabbits during other months of the year. A mature larva collected in June pupated, and the adult emerged in August; and others collected in November emerged as adults in March and April; thus indicating the existence of two generations a year in southern Georgia.

The percentage of hosts infested and the number of larvae in some of the hosts were surprisingly high. Five out of twelve cotton-tails collected near Valdosta, Ga., during November and December, 1936, were infested with warbles. Furthermore, five out of the seven uninfested rabbits had old scars or cysts, indicating previous infestation. Ten warbles were found in one of the infested rabbits. Of these only three were alive, but after the animal was skinned the seven other dead and encysted larvae in various stages of development were found, besides two recently evacuated cysts. A domestic rabbit, obtained at Valdosta, was found to be infested with nine living larvae and to have at least one evacuated cyst. Such high infestations are probably uncommon, but there is no doubt that *Cuterebra buccata* is abundant in the vicinity of Valdosta.

The larva of *Cuterebra buccata* seems to prefer to encyst in the region of the genital organs, especially in the scrotum of the male. Larvae have been found, however, in the skin over various other parts of the body except the head. The second and third instars, at least, are encysted wholly in the skin. Three dead first-instar larvae, apparently *C. buccata*, were found in the connective tissue between the skin and the underlying muscular tissue. One of these larvae was in the process of molting. No opening through the skin of the host was apparent in the vicinity of the first-instar larvae; but in one instance a second-instar larva that had not entirely discarded its first-instar exuvium had effected an opening through the skin of the host.

The length of the entire larval life has not been ascertained; but

since mature larvae have been collected from young rabbits estimated to be one-third grown, the developmental period appears to be relatively much shorter than that of such OESTRIDAE as *Hypoderma* and *Gasterophilus*. One observation on the duration of the second instar, from the time of appearance of the opening in the skin of the host until the molt to the third instar, indicated a period of from 4 to 5 days. The third instar in the same host required 10 days.

As stated, larvae collected in November pupated and emerged as adults in March and April, after pupal periods ranging from 103 to 129 days, whereas one mature larva collected in June pupated and emerged in August, after a pupal period of 75 days.

Cuterebra cuniculi (Clk.)

The larvae of *Cuterebra cuniculi* were taken from the marsh rabbit during March, October, November, and December. According to various laymen, this species of rabbit is also infested during the spring and summer months in the vicinity of Valdosta.

Cuterebra cuniculi has not been found in the cotton-tail in the vicinity of Valdosta, nor has the larva of *C. buccata* been found in the marsh rabbit. This indicates a high degree of host specificity.

Fifteen marsh rabbits were examined by the writers or reported on by the hunter who collected most of them. Twelve of these were infested with from one to three warbles. Nine infested marsh rabbits were observed by the writers, and in each case the warbles were encysted in the skin covering the ventral and lateral surfaces of the neck.

The pupal stage of *Cuterebra cuniculi* as determined for larvae collected in November and December ranged from 109 to 133 days.

Attempts to mate reared adults of *Cuterebra cuniculi* were unsuccessful, although infertile eggs were deposited by the females. The egg of this species is shown in Fig. 12.

LARVAL CHARACTERS

The present studies on the taxonomy of *Cuterebra* larvae have been limited to the second and third larval instars. Insufficient material prevents a discussion of the characters of the first instar.

All the species examined by the writers have the same general shape and structure in the respective larval instars, although differences are apparent in a detailed study.

The second-instar larva (Fig. 7) may be readily distinguished from the third instar (Fig. 1) by differences in the size, shape, number, and distribution of spines; the structure of the posterior spiracles; and the general appearance of the larva.

The characters that appear to be of most value in distinguishing species in the second larval instar are: (1) the relative size and shape of the

spines, (2) the presence or absence of spines on certain segments, and (3) the structure of the posterior spiracles. Other characters of lesser importance are the size and shape of the larva, the general structure of the cephalopharyngeal mechanism, and the size and shape of the anterior spiracles. The cephalopharyngeal sclerites may provide important diagnostic characters, but since a limited number of specimens from only a few species have been studied, their full worth cannot be determined at this time.

The spines and posterior spiracles again furnish the most useful characters for differentiating species in the third instar. The size and structure of the spines vary considerably in different species. In *Cuterebra buccata* and *C. cuniculi* practically all the spines have only a single point, whereas in other species the number of points ranges from 1 to 3, or even as many as 10 or 12 fine, comblike points may be present (Figs. 9–11). The posterior spiracular plates, in the various species examined, differ in general shape, size, and detailed structures (Figs. 2, 3, and 8).

Other characters apparently of value in differentiating the various species in the third instar are: (1) The number and distribution of spines on the segments, (2) the size and shape of the larva, (3) the size and shape of the anterior spiracles, (4) the size and general appearance of the cephalopharyngeal sclerites, and (5) the degree of pigmentation of the spines in certain regions of the body.

Owing to growth and other changes that take place, there may be a marked contrast in the general appearance of newly molted larvae and those that have reached the maximum growth in the same instar. At times these changes are confusing, especially to those unfamiliar with the larvae.

In larvae newly molted into the second instar the spines are generally closely approximated, and at this time the general appearance of the spiny armature differs considerably from that of older larvae when, owing to the growth of the integument, the spines are more widely separated. The spines themselves appear to increase in size somewhat owing to the deposition of pigment at the base, but this change is generally not marked. The structure, distribution, and number of spines, however, show no appreciable change. There is also no apparent change in the structure of the posterior spiracles in second-instar larvae, although the two spiracles become more widely separated in the older larvae of the same instar.

Newly molted third-instar larvae of *Cuterebra* are creamy white. As the larvae mature the spines become darker, and larvae near maturity are dark brown to black. The spines in newly molted third-instar larvae are also less widely separated than those in the mature larvae.

The posterior spiracles in the third instar undergo a gradual change in appearance such as also occurs in such well known species as *Hypo-*

derma lineatum (De Vill.) and *H. bovis* (Deg.) However, certain definite characteristics remain constant in larvae throughout any one instar.

Specific Larval Characteristics

Second-instar larva of Cuterebra buccata. Body more or less elongated. One newly molted larva measured 5.85 mm in length and 1.62 mm in width at the widest point. A fully grown second-instar larva measures approximately 10.5 mm in length and 4.5 mm in width.

The cephalic segment is small and is usually partly retracted. The two anterior lobes bear small sensory tubercles. A group of spines is situated ventrally in the region of the mouth.

Segment 2, which appears to be a part of the head segment, may or may not possess from one to three lateral spines. Segments 3 to 12 are well armed with dark, sharp, single-pointed spines. A band made up of several rows of posteriorly directed spines is present at the anterior margins of segments 3 to 10, the number of spines in each band decreasing posteriorly. A single row of spines, more or less in the central portion of the segment, is found on segments 3 to 7. On segments 3 to 5 these spines form a completely encircling row, whereas on segment 6 the row is generally interrupted on the dorsum. On segment 7 the spines are restricted to about four to six spines on each side and the venter may or may not be provided with several spines. These spines may be absent on segment 8, but if present are reduced to one or two spines on the sides.

The posterior margins of segments 7 to 12 possess anteriorly directed spines similar in size and shape to those at the anterior positions of the segments. On segment 12 all the posterior half of the segment is armed, whereas the spines on the more anterior segments are progressively reduced. On segments 9 to 12 the spines completely encircle the segment, although greatly reduced on the venter of segment 9. On segment 8 the spines are absent from the venter and greatly reduced on the dorsum or absent for a short space; and on segment 7 the spines are reduced to a single row of lateral spines. The inconspicuous anterior spiracular openings are situated one on each side and at the base of segment 2. The anterior spiracles are, as in the third instar, internal. The spiracles measure approximately 0.30 mm in length and 0.26 mm in width at the widest point.

The last apparent, or twelfth, segment is more or less truncate behind. On each side of the anus is a rounded anal lobe. The posterior spiracles are situated at the end and in the center of the segment (Fig. 6). The slits or spiracular openings show considerable variation in structure. The spiracle appears to be made up of two slits; the inner is more or less straight, whereas the outer is somewhat serpentine and may or may not be divided into two separate divisions.

The general appearance of the cephalopharyngeal mechanism is shown in Fig. 4. The length and width at the widest point are, respectively, 1.6 and 0.75 mm.

Third-instar larva of Cuterebra buccata. The length of four apparently mature larvae ranged from 26 to 32 mm, with the width approximately equal to one-half the length. The larva is widest at segment 7 and tapers to each end, segments 6 and 8 being only slightly narrower than segment 7.

The larva is armed with spines that are well distributed over the various segments (Fig. 1). In newly molted larvae the spines are without a dark pigment, but they gradually darken, becoming dark brown to black in the mature larvae. The spines, in 4 to 6 rows at the anterior portion of the segments, are provided with sharp single points (rarely two) that are directed posteriorly; similar spines on the posterior portion of the segment are directed anteriorly. The central rows of spines of each of the segments are somewhat flattened and are without well-defined points. The spines are shown enlarged in Fig. 9. The larger spines measure 0.40 to 0.45 mm in length from base to tip. The width of the larger spines at the base ranges from 0.30 to 0.35 mm. Small sensory papillae are situated on each body segment. These are spaced at regular intervals and range from 6 to 12 on each segment.

The first segment is small and generally partially retracted. It bears two large anterior lobes, each provided with two small sensory tubercles. The oral hooks generally protrude from the ventrally located mouth opening. The ventral side of the head segment, or first segment, is armed with several rows of small, almost colorless spines, whereas the dorsum is devoid of spines.

The openings to the anterior spiracles are situated on each side near the base of segment 2. The large spiracular structures are internal. The anterior spiracle, from the junction of the tracheae to the external opening, is 1.7 mm long, and the width at the widest point is approximately 0.9 mm. The anterior spiracles are everted during the formation of the puparium.

The twelfth segment (Fig. 2) is generally completely retracted in preserved specimens. On each side of the anal opening is a large rounded lobe. Below the center of the segment are four poorly defined tubercles while two are above the center. The two posterior spiracular plates occupy a central position on the last segment. They appear as two more or less kidney-shaped plates perforated by a number of sinuous spiracular openings. Each spiracle is divided into three distinct divisions, each probably corresponding to spiracular slits in such families of higher Diptera as Calliphoridae, Muscidae, and Gasterophilidae. In newly molted larvae the divisions are well separated but as the larvae mature the plates become darker and the divisions become less distinct

owing to the formation of dark pigments in the plate between and around the spiracular divisions. The scars produced by the discarded spiracles of the preceding larval instar are apparent between the spiracular plates. In many related families of higher DIPTERA this scar is well defined and is termed the "button." The spiracular plates are approximately 0.88 mm long and 0.45 mm wide.

The cephalopharyngeal mechanism is shown in Fig. 5. The dorsal cornu and ventral cornu are not pigmented in *Cuterebra.* In one specimen the greatest length and width of the cephalopharyngeal mechanism were 4 and 1.8 mm, respectively.

Third-instar larva of Cuterebra cuniculi. The larva of *Cuterebra cuniculi* is larger and slightly more robust than that of *C. buccata.* The length of apparently mature larvae ranged from 35 to 43 mm.

The distribution, general arrangement, and shape of the spines are similar to those of *Cuterebra buccata* (Fig. 1). There is a distinct contrast, however, in the size of the spines in the two species (Figs. 9 and 11). The larger spines in *C. cuniculi* measure from 0.55 to 0.65 mm in width at the base and from 0.65 to 0.80 mm in length on the longest side. The spines generally have only a single point, but occasional spines may have two points. From three to five rows of spines on the posterior margin of segment 11 are more or less of an orange color, even in mature larvae, which contrast with the almost black spines on other portions of the segment. In *C. buccata* the spines in this region are little, if any, lighter in color than on other portions of the larva.

The structure of the cephalic segment and the arrangement of spines thereon are similar to those described for *Cuterebra buccata.*

The anterior spiracles in two specimens ranged in length from 2.4 to 2.6 mm and in width from 1.7 to 1.8 mm.

The posterior spiracles are shown in Fig. 3. The three spiracular divisions are poorly defined in mature larvae. The general shape of the spiracular plates and the spiracular divisions will readily distinguish this species from *Cuterebra buccata.* The length and width of the posterior spiracles are approximately 1.1 and 0.5 mm, respectively.

The cephalopharyngeal mechanism, although larger, is similar to that of *Cuterebra buccata.* The greatest length and width in one specimen were, respectively, 5.5 and 2.7 mm.

Although the larvae of several undetermined species of *Cuterebra* were studied, detailed differences will not be mentioned. It may be stated, however, that specific larval characters are apparent in many if not most of the species of *Cuterebra.* For comparative purposes the posterior spiracular plates and the spines on a species taken from a chipmunk are shown in Figs. 8 and 10.

Explanation of Plates

Plate I

Fig. 1. Lateral view of third-instar larva of *Cuterebra buccata;* ×4½.

Fig. 2. Posterior view of twelfth segment of third-instar larva of *Cuterebra buccata,* showing the posterior spiracular plates and spiracles; *b,* button; *st,* sensory tubercles; *al,* anal lobe; ×15.

Fig. 3. Posterior view of twelfth segment of third-instar larva of *Cuterebra cuniculi,* showing the posterior spiracular plates and spiracles; lettering as in Fig. 2; ×15.

Fig. 4. Lateral view of right half of cephalopharyngeal skeleton of second-instar larva of *Cuterebra buccata;* ×50.

Fig. 5. Lateral view of same, third-instar; *oh,* oral hook; *h,* hypostomal sclerite; *da,* dorsal arch; *vc,* ventral cornu; *dc,* dorsal cornu; ×20.

Fig. 6. Posterior view of twelfth segment of second-instar larva of *Cuterebra buccata,* showing the posterior spiracular plates and spiracles; ×16.

Fig. 7. Lateral view of second-instar larva of *Cuterebra buccata;* ×4.

Fig. 8. Posterior spiracular plates of third-instar larva of *Cuterebra* sp. from a chipmunk; ×15.

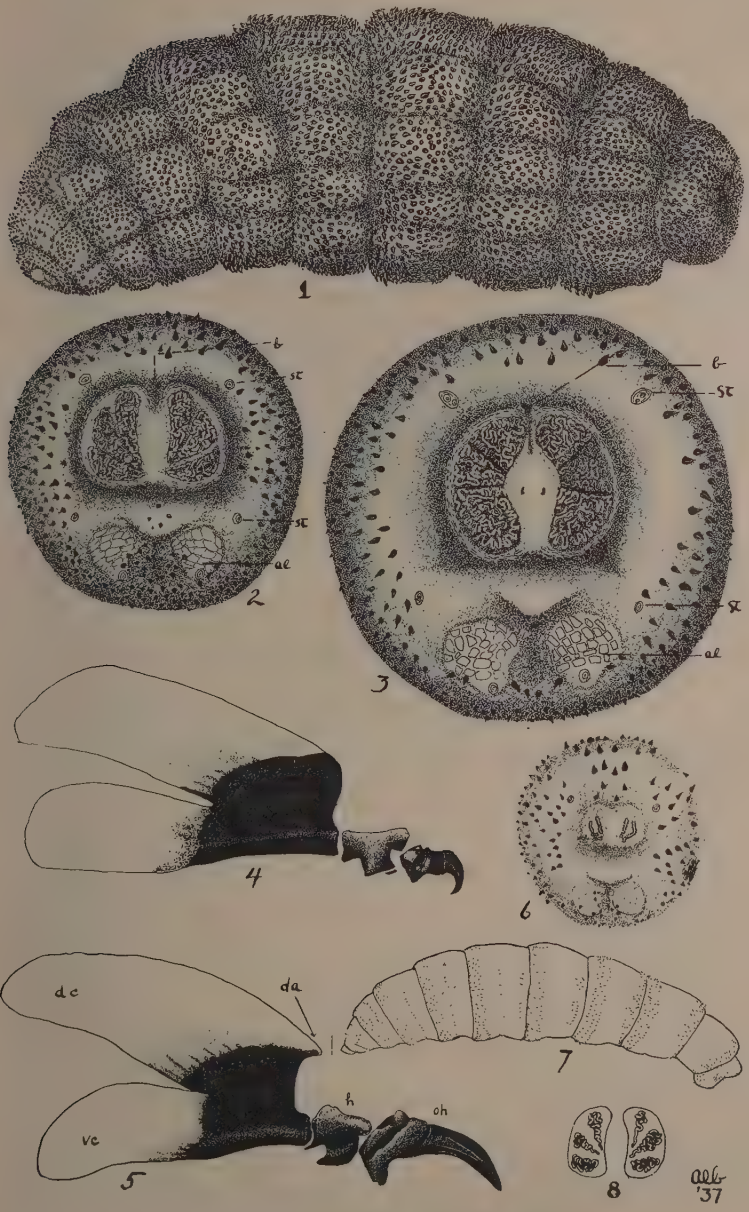
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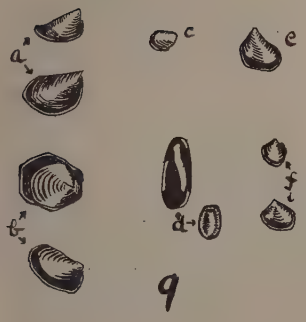
PLATE II

FIG. 9. Spines on the skin of third-instar larvae of *Cuterebra buccata:* First row, posteriorly directed spines found in the anterior 4 to 6 rows on each segment, *a,* side view, *b,* top view; center row, rounded to flat spines found in the center rows on each segment, *c,* side view, *d,* top view; third row, anteriorly directed, sharp-pointed spines found in the posterior 3 to 4 rows, *e,* side view, *f,* top view. All × 30.

FIG 10. Spines on the skin of *Cuterebra* sp. taken from a chipmunk: First row, from the posteriorly directed anterior rows of spines, *a,* side view, *b,* top view; center row, central flat spines, *c,* side view, *d,* top view; third row, from anteriorly directed posterior rows of spines, *e,* side view, *f,* top view. All × 30.

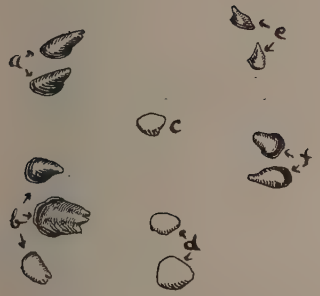
FIG. 11. Spines on the skin of larva of *Cuterebra cuniculi:* First row, from the posteriorly directed anterior row of spines, *a, b,* side view, *c, d,* top view; center row, spines from the center row, *e,* side view, *f, g,* top rows; third row, from the anteriorly directed posterior row of spines, *h, i, j,* side views, *k, l,* top views. All × 30.

FIG. 12. Outline of side view of egg of *Cuterebra cuniculi, op,* operculum. × 24.



9

11



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all
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STUDIES UPON *STRONGYLUS VULGARIS*. IV. A METHOD OF DETERMINING THE ENDPOINT OF THE REACTION OF A LETHAL AGENT AGAINST ADULTS IN VITRO*

J. H. Whitlock

Although numerous investigators have attempted tests with various lethal agents against helminths in vitro, almost universally the criterion of death has been the cessation of movement. This is essentially a negative factor, the determination of which is extremely difficult with any degree of accuracy. In previous work with *Strongylus vulgaris* (Whitlock, Link, and Leasure, 1939) cessation of movement was used as the criterion of death, but it was noted at the time that these parasites eventually developed a true rigor mortis after movement ceased. This fact led to the development of the technic and apparatus herewithin described, by which it is possible to determine the time of death by a positive rather than a negative character.

At first pressure with a bent dissecting needle was used for the determination of the death of the parasite. If the parasite's body was comparatively hard it was considered dead. However, since a time-action experiment with *S. vulgaris* required observations at times when the observer was noticeably fatigued and since the observer was troubled by a constant desire to escape from the highly humid, uncomfortable environment of the incubator in which the worms were kept, it was obvious that the relative accuracy of observation could be obtained only with the development of a mechanism which left as little as possible to the human element in the determination of the death time.

With the advice and cooperation of Professor Gabe Sellers of the department of shop practice of Kansas State College a suitable instrument was developed. Fig. 1 is a diagrammatic drawing of this mechanism. Essentially it consists of a flat piece of copper (ABC) to one end of which is soldered a hook (A) and to the other an electric contact (C). This whole structure is suspended by an axle at point B from the metal case (E) for the instrument. At point D a piece of wire spring steel 0.5 mm in diameter is soldered to the shaft (ABC). By means of the set screw (F) which is attached to the metal case, tension on the spring can be adjusted so very delicately that within the space limits involved it is possible to adjust to a tenth of a gram the amount of pressure upward on the hook (A) necessary to make a contact of C with H. H is

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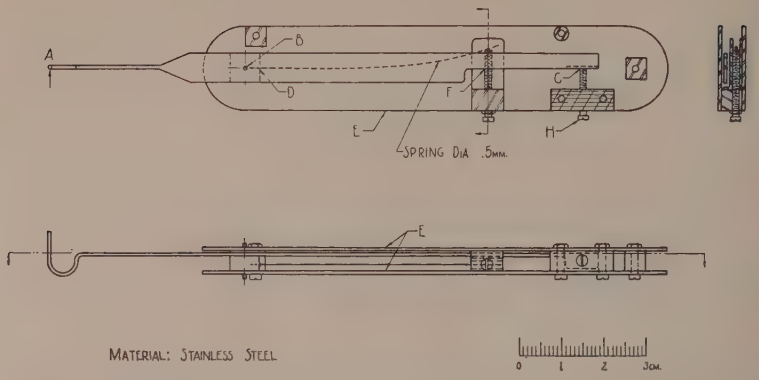

FIG. 1. Showing three views of the rigor-mortis instrument: a plan, elevation, and cross-section as indicated by the arrows.

a metal bolt set in a fiber block, hence insulated from the rest of the instrument; by decreasing or increasing the distance between it and C another adjustment of the instrument is possible. One pole of a battery is attached to H and the other is connected in series with a telephone receiver to the metal case of the instrument.

Calibration, by means of a torsion balance, is always done at the temperature at which the instrument will be used in the incubator. One tray of this balance has a sharp ridge projecting above its center, in the other is placed the desired weight. By means of attaching the instrument to the substage condenser frame of an old microscope and by use of its rack and pinion gear the exact center of the hook is delicately brought into contact with the projecting ridge in the balance pan. The screw F is adjusted to such a point that a small amount of downward pressure with the hook will cause a vibration in the earphone reflecting the vibration of the electric motors in the incubator. For calibration, then, the instrument is really used as a type of seismograph.

The instrument used in the experiments recorded in article V of this series (Whitlock, 1940) was made entirely of copper, but any further instruments of this type which may be constructed are recommended to be of stainless steel since corrosion of the copper interferes with the usefulness of and shortens the life of the instrument.

To determine the degree of rigor mortis of a specimen of *S. vulgaris* the instrument is applied at an angle of approximately 20 degrees to the parasite. If the body bends before the click caused by contact of C with H occurs in the receiver, the parasite is considered to be alive; if the body is still resisting the pressure when the click occurs, the parasite is considered dead. Experience has shown that the instrument will eliminate most of the parasites still capable of movement but with a hard surface,

if it is calibrated against a direct perpendicular pressure of 4.7 gms. Since, however, the instrument in practice is applied at an angle the actual force resisted by the dead worms is found by multiplying the calibration force by the cosine of the angle at which the instrument is applied. (In this case 4.4 gm).

There is a possibility that the use of this instrument introduces a systematic error into time-action experiments especially at the beginning and end of such tests. At the beginning of a time-action experiment occasionally a few worms are found which are determined dead by the instrument, but which are revivable for a short period of time. At the end of this type of experiment a specimen of the parasite is sometimes found which has never shown any evidence of rigor mortis, but which simply disintegrates. If death be defined as the production of a certain degree of rigor mortis, this source of error is minimized. It is hoped that it will be possible in the future to measure and define the error more exactly. However, even with the systematic error introduced, since it appears to be constant, the author believes that the use of the rigor mortis instrument for determining death allows of far greater accuracy than any method previously proposed.

SUMMARY

An instrument is described which measures with a fair degree of accuracy the appearance of rigor mortis in *Strongylus vulgaris* during tests in vitro, and hence makes an excellent criterion of death. While such a method is not perfect, it permits of greater accuracy than any other method applicable to helminths proposed to date.

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STUDIES UPON *STRONGYLUS VULGARIS*. V. IN VITRO TOXICITY OF COPPER SULPHATE AND THE CUPRIC HALIDES[1]

J. H. Whitlock[2]

Although copper salts are such effective poisons for the lower forms of life, little work has been done with any of the copper compounds as anthelmintics, except for the extensive investigations of copper sulphate in trichostrongylid infections in sheep and some recent reports on the efficacy of certain proprietary copper anthelmintics. Because of this the action in vitro of a number of common cupric salts was tested against *Strongylus vulgaris*. The present paper reports the toxicity of cupric sulphate, chloride, fluoride and bromide for this parasite.

MATERIALS AND METHODS

The specimens of *Strongylus vulgaris* were collected from horses at the Topeka plant of the Hill Packing Company and transported to Manhattan in a thermos bottle containing a physiological saline solution (either Ringer's or 0.85 per cent NaCl) heated to a temperature of 38° C. Since it has been shown (Whitlock, Link, and Leasure, 1939) that the male and female *S. vulgaris* vary in susceptibility to poisons, only females were used in these experiments. Upon arrival in Manhattan the worms were counted in groups of fifty into fingerbowls containing warm 0.85 per cent NaCl solution and left in an incubator for from one to three hours. During this time the test salts were dissolved in 0.85 per cent NaCl solution at 20° C; then the solutions were heated to 37–38° C and kept in the incubator until needed. Cupric fluoride was the only salt used which was not easily soluble in water. In order to make the data obtained from testing cupric fluoride comparable with the data from the other salts, the cupric fluoride solutions are reported in degrees of molarity. But it should be understood that in this case "molar solution" merely means the ratio between the salt and the solvent since even in a 0.0033 molar preparation all of this salt did not dissolve. In preparing these mixtures the amount of salt necessary to make the proper degree of

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[2] The author is deeply indebted to Dr. G. N. Reed of this station for advice on some of the chemical phases of the work and to Drs. E. E. Leasure and R. P. Link for the loan and standardization of the potentiometer used in these experiments. The author also wishes to acknowledge the great assistance rendered him by the staff of the Hill Packing Company of Topeka, Kansas, and the officials of the U. S. Bureau of Animal Industry on meat inspection service at that plant in collecting the parasites.

molarity was added to the 0.85 per cent NaCl solution and vigorously shaken. When saturation was complete, the preparation was applied to the worms as a mixture of dissolved and suspended cupric fluoride. All the chemicals used were of the chemically pure grade except the sodium fluoride, the standard of purity of which was not available.

When the time came to test the worms, the saline solution was poured off and the parasties washed once with the test solution; then 100 cc of the test solution was poured on the worms and they were placed in a slow shaker in an incubator for 100 minutes. The time of the first exposure to the poison was considered the beginning of the test. When the test period was over, the worms were washed in a neutral carbonate or phosphate-containing saline solution and placed twenty-five at a time in the special culture dishes already described (Whitlock, 1939), and a slow gravity drip of a saline solution[3] started into the dishes.

The arrangement of the apparatus differed from that previously described (op. cit.) in that the small incubator was eliminated. The temperature of the drip solution was regulated by passing it through a coil in a water bath, the heater under which was controlled by a thermostat over which the drip solution subsequently passed. The drains were made of horizontal pieces of garden hose in which holes had been bored to receive the small end of the special culture dish. In Test 7 (see experimental data) the fluid in the drains was run off by gravity instead of by a vacuum pump as in the other tests. Since the former method does not circulate as much air over the culture dishes, it causes less evaporation and thus gives better temperature control. In the first six tests the temperature of the drip solution in the different culture dishes varied between 35° and 38° C. In Test 7 there was approximately 0.5 degree variation from the average temperature of 38° C. It was found that a lip approximately one centimeter high is more convenient than the larger lip on the culture dishes figured in the previous paper (op. cit.).

Preliminary tests indicated that the action of the copper salts tested was distributed in logarithmic time intervals; hence the time-mortality data were obtained by observing and recording the numbers of dead worms at logarithmic time intervals. Rigor mortis determined by the instrument already described (Whitlock, 1940) standardized to a perpendicular force of 4.7 gm was used as the criterion of death in these experiments.

For clarity in subsequent discussion a test group is defined as a group of approximately 100 worms subjected to the action of a test salt. A test is defined as the study made of the reaction of a number of test salts

[3] The saline solutions used were (1) simple Ringer's neutralized with $NaHCO_3$ or strained cecal fluid, (2) 0.85 per cent NaCl neutralized with $NaHCO_3$ or (3) Britton and Robinson's universal buffer (using acetic acid) plus 0.65 per cent NaCl (Britton, 1931).

on a number of test groups, the experimental animals of which were collected at the same time and under the same conditions. Each test always contained a control group of 60 to 100 worms which were exposed to the action of the 0.85 per cent sodium chloride solution used to dissolve the test salts and a copper sulphate test group. Each test should be considered separately since the average susceptibility to copper poisons varied with each test. However, the relative toxicity of the different compounds may with justification be compared in the several tests.

The data obtained from these experiments were analyzed according to the method of Bliss (1937). This method consists of converting the numbers of worms dead at each time interval from each test group to cumulative percentages and then to probits, units which convert the normal probability curve to a straight line. When the probits were plotted against the logarithms of the time intervals, a line, straight for approximately 85 per cent or more of the action was usually obtained. By inspection of this line it was then possible to determine the mean lethal time (LT50) and the standard deviation. The standard error of the mean was calculated by the formula $sm_1 = s_1/\sqrt{n}$. Calculation of the mean or correction for biological or experimental truncation was not done because the graphic estimate could be obtained with a fair degree of accuracy and the variation between the different tests was too extensive to warrant further correction. In some test groups there was a noticeable tendency for the plotted points to fall along a slightly convex line, but no other function of the time than the logarithmic gave a consistently better fit. The data from those test groups whose mean lethal time (LT50) was above 1000 minutes (especially the control groups) were sometimes difficult to fit to a straight line, hence these mean lethal times are not always as accurate as the others.

At this point it would be well to emphasize that the use of Bliss' method of analyzing time-mortality data in this case as in a previous study (Whitlock, Link, and Leasure, 1939) in no instance gave rise to a conclusion unsupported by any other accepted type of analysis of the experimental data. It sums up in a convenient *and accurate* shorthand the large number of facts recorded as part of a time-action experiment.

In this paper the mean lethal time or time necessary to kill half of the worms in a group is recorded as LT50, a term analagous to the LD50 used by pharmacologists and insect toxicologists for the mean lethal dose. Thus in Test 2 in Table 1 it is recorded that the LT50 for a 0.01 molar solution of copper sulphate is 660 $\pm$ 35 minutes. In other words, this is the time determined by graphic analysis of the data at which 50 per cent of the worms in this test group died. One should beware of assuming, however, that the standard error as given with this or any other LT50 in this paper represents the total limits of error in these experiments. A systematic error is introduced in these tests by (1) the concurrent lethal

effect of the low hydrogen ion concentrations and (2) the use of the rigor mortis testing mechanism. No satisfactory method of measuring these sources of error has yet been devised. The accidental error is at least five per cent (Clark, 1933), and may be double this figure, since the accidental error of the volumetric and observational methods in addition to fatigue error must be considered.

Throughout these experiments a constant check was kept on the pH of the solutions, using a quinhydrone potentiometer standardized against an acetate buffer.

EXPERIMENTAL DATA

Problem 1: To determine the relative toxicity of copper sulphate and the cupric halides for *Strongylus vulgaris* in vitro.

Table 1 gives the summation of the results of a series of seven tests chiefly with 0.01 molar solutions of copper sulphate and cupric chloride, bromide, and fluoride.

Study of the rapidity with which death is brought about by these various compounds as indicated by the LT50 for each experimental group shows:

1. That copper salt solutions are lethal for *S. vulgaris* in vitro, since the LT50s for these solutions are consistently lower than the LT50s for the comparable control groups.

2. That copper sulphate and cupric bromide and chloride are approximately equal in toxic effect since in tests 4, 5, and 6 the relative toxicity of each compound in each test is approximately the same.

3. That cupric fluoride is more toxic than any other of the salts studied as is shown by the fact that only in Test 7 in which a 0.0033 molar preparation of this compound was used was it less effective than the other copper compounds.

4. That cupric fluoride is more erratic in action than any other compound studied as is shown by the inconstancy of its relative toxicity when comparing it with copper sulphate in the first five tests recorded in Table 1.

Problem 2: To determine why cupric fluoride is the most effective poison of the salts tested.

The most logical explanation of the greater toxicity of cupric fluoride for *S. vulgaris* lies in the probability of a synchronous toxic action of the copper and fluorine ions. To determine if the fluoride anion is toxic for *S. vulgaris* a 0.01 molar solution of sodium fluoride was applied to 100 worms as parts of Tests 4 and 7. Analysis of the time action data gave an LT50 of 575 $\pm$ 27 minutes in Test 4 and 646 $\pm$ 27 minutes in Test 7. Referring to the cupric sulphate groups for these tests in Table 1 it will be noted that the LT50 values for sodium fluoride is roughly equivalent in effect to them. Since this is true a 0.0033 molar solution of cupric

TABLE 1.—*Giving the relative toxicity of 0.01 M solutions of simple cupric salts for* S. vulgaris *exposed to their action for 100 minutes as measured by the mean lethal time of the reaction (LT50)*

Test	$CuSO_4 \cdot 5H_2O$	$CuF_2 \cdot 2H_2O$	$CuCl_2 \cdot 2H_2O$	$CuBr_2$	Control	Type of drip solution
pH Range of .01 M Sol ...	3.65 – 3.93	3.95 – 4.35	3.55 – 3.73	3.34 – 3.70	6.23 – 6.75	
Range s_i (Log time)	.14 – .26	.10 – .23	.11 – .22	.16 – .22	.11 – .24	
LT50 Test 1 (Min)	660 ± 35[1]	313 ± 9			1122 ± 37	Strained cecal content 1 part; simple Ringer's 8 parts
LT50 Test 2 (Min)	562 ± 30	251 ± 10			1027 ± 59	Modified Buffer
LT50 Test 3 (Min)	631 ± 20	417 ± 2			1259 ± 89	Modified Buffer
LT50 Test 4 (Min)	646 ± 30	251 ± 13	660 ± 27	631 ± 25	1514 ± 70	Modified Buffer
LT50 Test 5 (Min)	501 ± 16	339 ± 4	479 ± 12	457 ± 16[2]	955 ± 97	0.85% NaCl & $NaHCO_3$
LT50 Test 6 (Min)	489 ± 30	347 ± 8[3]	490 ± 27	513 ± 26	1148 ± 53	Ringer's + 0.05% $NaHCO_3$
LT50 Test 7 (Min)	478 ± 20	562 ± 49[4]			1349 ± 47	Ringer's + 0.05% $NaHCO_3$

[1] Based on 68 worms.
[2] Mean estimated with difficulty.
[3] 0.005 M solution.
[4] 0.0033 M solution.

fluoride should have approximately the same effect as a 0.01 molar solution of copper sulphate, and it does as is indicated by Test 7 of Table 1.

That there is no more than a rough agreement between the expected and actual results is due to the activity of a number of factors in addition to the experimental error. First: the time-concentration relationship is seldom simply linear; it follows the formula $C^n t = \text{Constant}$. The value of n for the effect of cupric salts upon *S. vulgaris* has not been determined, but on the basis of results obtained by investigators working with other small experimental animals (Clark, 1933) n is probably less than one. The values of n for fluorine were not available. Second: all of the cupric fluoride was not in solution. Third: the experiments with sodium fluoride give some indication that the fluoride ion may be erratic in its action, although this may be due to the fact that this chemical was of dubious purity.

Problem 3: To determine why cupric fluoride is the most erratic in action of the various salts studied.

The simplest and most obvious difference between cupric fluoride and the rest of the cupric halides lies in its relative insolubility. Some of the salt always dissolved, but in every test with this compound some of the salt failed to go into solution. To see if the solvation of a compound has any effect upon its toxicity, in Test 3 one group of 100 worms was exposed to the amount of cupric fluoride necessary to make a 0.01 molar solution; another group of 99 worms was exposed to some of this same mixture which had been filtered. The death rates and LT50s for the two groups were identical, hence these data are combined in Table 1. This proves that only the salt which is actually in solution is effective as a poison.

In none of the standard handbooks of chemistry is the exact solubility of cupric fluoride listed. Such lacunae are invariably due to the inconstant solubility of the salt in question. Since in every test with this salt we were dealing with a saturated solution and since the amount of this salt necessary to make a saturated solution apparently is variable and since we have shown that it is the salt actually in solution which is toxic, it would be logical to conclude that much of the variable relative toxicity of this salt is due to its variable solubility. There is some evidence, however, to indicate that this is not the only factor operating since sodium fluoride tests as reported in Problem 2 are not constant in relative toxicity.

Problem 4: To determine why cupric salts are effective against *S. vulgaris* in vitro but not in vivo.

The most obvious answer to the question posed lies in the fact that cupric salts are precipitated when they come in contact with neutral solutions. Since much of the neutralization of the acid content of the stomach is due to the action of the carbonate salts secreted by the small

intestine, it was determined to study the effect of toxic cupric salts precipitated by sodium bicarbonate. These investigations were carried on as parts of Tests 2, 3, and 7 (see Table 1), and the resulting data are given in Table 2. It will be seen from a study of these tables that 0.01 molar

TABLE 2.—*Showing the relative inertness of cupric salts when precipitated by sodium bicarbonate: parts of Tests 2, 3, and 7 given in Table 1*

Test	Salt	LT50	pH
Test 2	0.01 M $CuSO_2$ ppt. by 0.1% $NaHCO_3$	1259 ± 41	5.84
Test 3	0.01 M CuF_2 ppt. by 0.1% $NaHCO_3$	1149 ± 53	6.20
Test 7	0.0033 M CuF_2 ppt. by 0.1% $NaHCO_3$	1318 ± 67	6.23

preparations of both cupric sulphate and fluoride are inactivated when precipitated by an excess (0.1 per cent) of sodium bicarbonate. The inactivation of cupric fluoride is somewhat illogical since it may be supposed that it involves the formation of a 0.02 molar solution of sodium fluoride which should be toxic for the worms. An explanation of this phenomenon is to suppose that the fluoride ion is also inactivated by sodium bicarbonate. To see if this is true, as part of Test 7 0.1 per cent sodium bicarbonate was added to a 0.01 molar solution of sodium fluoride and the resulting solution tested as were the others. The time-action data gave an LT50 of 1122 ± 52 minutes which proves that the toxic action of sodium fluoride is inhibited by the presence of an excess of carbonate. Although no precipitate was formed by this reaction, the pH of the solution was 6.89 compared with 5.84 and 4.24 (this 4.24 reading may be erroneous because trouble was experienced with the potentiometer in the test), the pH values of 0.01 molar solutions of simple sodium fluoride in Tests 4 and 7 respectively. From these data it may be concluded that fluoride as well as copper ions are prevented from toxic action in vitro, and probably in vivo, by the presence of an excess of carbonate ions, although in all probability the essential reaction responsible for the inhibition differs since a precipitate is formed in one case and not in the other.

DISCUSSION

From these experiments the conclusion may be reached that the toxicity of simple copper compounds for *Strongylus vulgaris* in vitro (and in all probability for other nematodes in vivo as well) is regulated by two factors: (1) the amount of soluble copper available and (2) the toxicity of the anion joined with the copper ion.

The amount of soluble copper in the host animal's gastro-intestinal tract derived from simple copper compounds and available for anthelmintic purposes is probably correlated inversely with the amount of carbonate present. Hence simple copper compounds will be effective as

anthelmintics only in acid environments such as the abomasum. There any insoluble carbonate formed is dissolved by the excess of acid, the active cupric chloride resulting. This is probably the explanation for the fact that copper sulphate solutions are markedly effective only against *Haemonchus contortus*.

Toxic anions supplement in an effective manner the action of the copper ion in vitro. In the present series of tests cupric fluoride was roughly three times as effective as cupric sulphate, an expected result since the toxic action of one active fluoride ion was roughly equivalent to the toxic action of one active copper ion. The fluoride ion, however, is apparently inactivated by carbonate salts although the mechanism whereby this occurs probably differs from that inhibiting the copper ion. As tested under the conditions of this study, it was also erratic in action. These facts in conjunction with the fact that the use of fluorine compounds as an "anthelmintic ration" has been patented as a live-stock remedy (U. S. Patent No. 2027967) make any study, except a purely incidental one, of the fluorides of doubtful value.

Attention should be called to the marked consistency that the time-action experimental method obtained when testing the relative efficacy of cupric sulphate, chloride, and bromide. Whether this apparent accuracy will continue with future tests is, of course, uncertain, but if so it will provide a valuable method of comparing possible anthelmintic compounds before expensive tests with host animals are begun.

Most cupric compounds react with carbonates to form a precipitate, which is toxicologically inert. Hence they may be eliminated from consideration as anthelmintics for oral administration against other than gastric parasites, unless they liberate an actively toxic anion when forming copper carbonate. Certain exceptions to this rule are being studied in this laboratory at the present time. An attempt is also being made to determine if the same objection will eliminate copper compounds from consideration as anthelmintics for parenteral administration.

These experiments permit a few predictions as to the properties of a more universally effective copper-containing anthelmintic, if such exists.

(1) It must be water soluble, or, according to Chitwood (1938), lipoid soluble and resistant to precipitation by buffer salts.

(2) It probably should be fortified by the possession of toxic anions which should remain effective even if the copper ion is precipitated by carbonates.

SUMMARY

1. The toxicity of simple copper compounds for nematode parasites varies with (a) the amount of soluble copper available and (b) the toxicity of the anion joined with the copper ion.

2. The fact that simple inorganic copper compounds can remain in

their effective soluble form only in an acid portion of the digestive tract affords a rationale for the effective action of copper sulphate against *Haemonchus contortus* in the abomasal lumen and its ineffectiveness against parasites in a more neutral environment.

3. The time-action experimental method offers definite promise in anthelmintic experiments in vitro.

4. A prediction is made of the properties of a more universally effective copper-containing anthelmintic as revealed by these experiments.

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PNEUMONYSSUS CANINUM N. SP., A MITE FROM THE FRONTAL SINUS OF THE DOG*

WALLACE L. CHANDLER AND DAVID S. RUHE

In February, 1937, a male Boston bulldog with a clinical picture of nephritis complicated by prostatitis was killed and autopsied in the Animal Pathology Laboratory at Michigan State College, East Lansing. Dr. Lloyd B. Sholl, who conducted the autopsy, upon splitting open the skull to examine the brain, discovered a considerable number of mites inhabiting the frontal sinuses of the dog. Miss Mae Clifford, a student present at the autopsy, brought two specimens of the mite to the attention of the senior writer, who identified them as dermanyssid mites belonging to the subfamily HALARACHNINAE (Banks). Dr. Henry E. Ewing's key to the DERMANYSSIDAE placed the mite anatomically close to the genus *Pneumonyssus,* with some linkage to the *Halarachne* species. The unusual site of the infection prompted further studies, and a description of the new species resulted.

The body of the animal, although discarded for several hours, was recovered and a study of the head, trachea and lungs permitted a very thorough search, by dissection and maceration of the parts. Examination showed the mites actively dispersing throughout the entire nasal region of the cold carcass. One mite only was found upon the lung surface. This one may have been transferred by either the hands of the autopsyist or later examiners, or possibly may have migrated there. From the sinuses and nasal passages of the animal, however, we collected 42 specimens of the mite. This was several hours after the death of the dog. In spite of careful search, due to the dispersal of the mites from their undoubted original location in the frontal sinuses, we may have lost many valuable specimens.

The specimens were killed by submergence either in very dilute colloidal iodine (Chandler) or in lacto-phenol, then mounted in arsenious-acid-glycerin-jelly, beneath a cover glass, for study. In the experience of the senior author this method has been satisfactory for mites if examined within a few weeks after being mounted, they have not, however, been well preserved by this method. Staining of the mites with borax carmine has been satisfactory only in revealing the anal and stigmal plates; dehydration and clearing in xylol showed no additional structures. Distortion and the production of confusing wrinkles also resulted. A full key of diagrams is included to illustrate the points of identification, along with a corresponding key of photo-micrographs. These latter have in

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a few cases been retouched with an etching needle, but at no time was this retouching used upon the significant portions of the photographs.

Pneumonyssus caninum, n. sp.

(Adult female)

(Figs. 1–18)

The general body shape is oval; gravid females bearing fully developed embryos are not greatly distended. Length of adult females 1.0–1.5 mm, average 1.15 mm, width at maximum 0.6–0.9 mm measured from the region posterior to coxa IV, average 0.52 mm. Color when living a very pale yellow. The exoskeleton, or cuticle, is smooth, moderately tough, with scant setae, no papillae and no conspicuous systematic striae.

The *capitulum* is quadrangular, slightly narrower at the top than at the base, one and one-half times as long as broad; 0.15×0.11 mm.

The *palpi* consist of five segments, considerably reduced in size; being less than the width of the capitulum in total length. The spineless basal segment is twice as broad as long ($20 \times 40\ \mu$), the second segment of the same proportions ($15 \times 30\ \mu$) and bears dorsally three very short conical setae, the third segment more elongate ($15 \times 22\ \mu$) with one dorsal seta, the fourth segment with a single ventral spine, elongate ($20 \times 15\ \mu$) while the tip segment is even more lengthened ($17 \times 11\ \mu$), crowned with a long tactile bristle of $25\ \mu$ arising on the inner dorsal surface and with a shorter, slenderer bristle arising on the opposite edge; 8–10 cone setae surround the tip of the palp. In the living mite the palpi are capable of considerable protrusion beyond the edge of the body. Total length of the palps varies from 75 to $80\ \mu$ or slightly less than the width of coxa I.

The *chelicerae* are well developed and clear cut, without setae, consisting of blunt, stylate chelae, the inner member of which is probably fixed and apparently fused to the cheliceral stalk, the outer member long-triangular, movable, possessing a lone blunt spine halfway up its external edge. The base of the external chela is broad and possesses joint surfaces articulating with the cheliceral stalk, so that there is both an inner and outer elbow. The chelicerae are capable of moderate extension from the mouth cavity.

The *hypostome* is bifid, the longitudinal division extending posteriorly completely to the fold that demarcates the thorax; there are corneous thickenings on the anterior and outer edges, these merging into the thin and indistinct lingulae that ventrally cover the chelicerae. Each lingula extends directly inward from its outer corneous edge, forming a knob directly over the chelicerae, dipping into a u-depression and rising to a cone junction with the other member of the hypostome.

The *epistome* is seen as a broad structure, bracket-shaped at its anterior face, merging into the capitulum.

The *dorsal plate* is fairly well-developed, coriaceous, covering a moderate area between the edges of the dorsum; it is unevenly sclerotized, of irregular pattern, but roughly leaf-shaped. Such irregularity has been ascribed to uneven muscle traction upon the plate in question. The anterior end is somewhat flattened, the posterior end divided. Anteriorly the plate extends to within $70\ \mu$ of the dorsum edge, extending partially over the mouth parts, posteriorly then for a considerable distance ($260\ \mu$) out over the abdomen. There are four pairs of inconspicuous setae, these corresponding in position to the four pairs of legs, and occurring toward the outer edges of the dorsal plate. Over all, the plate is studded with microscopic, back-curving spines.

The *peritremata* are maul-shaped, uneven and shortened from less highly parasitic forms, such as the common *Liponyssus* species parasitic externally on fowls. They have well sclerotized walls, within which the spiracular openings are visible as oval pores leading directly into a spherical trachea, from which branch an undetermined number of tracheoles. These cannot be seen accurately and require special technique. The spiracles have long, probably non-functional slits extending anteriorly, grooving the peritremes. All the remains of the spiracle rakers (so fre-

quently seen in ACARINA) are fine flutings observable thruout the length of both spiracle and groove. There is found, inconstantly, a line leading from the peritremes to behind the fourth coxa. Since a tracheole parallels this fold it is possible that this is an anatomical artefact, here and in kindred species where it has been attributed a significance. The peritremes are laterally placed, above and between coxae III and IV, the spiracle bulb being located most posteriorly, the stem of the peritreme curving anteriorly and dorsally; they measure, on an average adult, 16 mm. The stigmal plates are long (0.23 mm) with an ameboid outline and surround the peritremata. Stained specimens alone reveal these plates.

The *tarsi* are slender, adapted for fairly active movement. Each consists of seven segments, a normal segmentation. The first pair of tarsi is equipped at the tarsus tip with a pair of heavily chitinized, hollow brown claws, adapted admirably for gripping. The pulvillus shows as a low knob between the claws. A spoon-like, brown, chitinous socket-piece deep in the tarsus tip, provides articulation for both claws. This piece shows irregularity to suggest that it may be fused, originally homologous to the counterpart structures upon the other tarsi. Long, special-sensory setae are found upon the first leg, and here upon the dorsal and anterior faces only. These may correspond in function to the Haller's organ of the tick, for there is no marked cluster of spines upon tarsus I; short cone spines are present, but scattered and in no significant grouping. The posterior tarsi show these short spines as lengthened, so that their special function upon the first leg may be implied. The latter three pairs of tarsi are more specifically those of walking. They are each tipped with a long, stalked sucker ambulacrum, armed with two slender hooks below a caruncle of undefined lobulation. Several specimens suggest two lateral lobes and a broad central lobe, but this is ill-defined. The hooks are set into a complex joint at the base of the suction cup, the joint consisting of three articulating pieces, the two basal of which are incurved, round-headed, and which articulates at the inner faces of these club heads. There is a basal coxa, a short trochanter, longer femur, shortened tibia and protarsus, and a tapering tarsus. The legs vary in total length, progressively lengthening from I to IV. Setae are scant on all but the tarsal segments; there is a slight numerical superiority in setae or dorsal and anterior location against ventral and posterior.

The *anal plate* is differentiable merely as a ring or rough oval of very lightly sclerotized exoskeleton surrounding the irregular anal slit. It is roughly ovoid, owns a triad of long setae, two lateral and anterior to the anus, the third slightly shorter and posterior to the slit. The anal plate is ventral, situated some distance anterior to the posterior body edge. The anus itself is, in non-gravid specimens, like a button cut longitudinally by the anal opening.

The *vulva* of these females is a well-demarcated transverse slit running across just behind the fourth coxae. There are definite heavy labial bands on both edges of the opening, and these articulate with each other near the bases of the fourth coxae by means of two angular, brownish, sclerotized shoulders. In one specimen the slit is gaping wide, revealing an orifice of 0.2 mm from shoulder to shoulder.

No *sternal plate* has been seen in the mature specimens. The larval forms clearly show a sternal "edge" bounding the region laterally but this runs from capitulum to abdomen, and from coxae to coxae, an extent greater than any similar plates of related acarina. Some adult mites show bounding folds without special thickening. The heavy sternal muscles radiating to the legs from the thorax ventrum are plainly seen, but there is no anchoring plate.

Setae are few and inconspicuous, as in the internal-parasitic mites. Dorsally and ventrally they are invariably paired, the dorsal thorax with two pairs not on the dorsal plate, these above the III and IV coxae respectively, the dorsal abdomen with four sets occurring linearly backward to a prominent pair projecting from the posterior body edge; the ventral thorax has three pairs, these approximating the gaps between the coxae, the ventral abdomen three additional sets projecting at regular intervals up to the posterior margin of the abdomen. All are set in papillae buds, prominent under high magnification, are stiff and generally curving, and point backward.

The *internal organs* of thorax and abdomen could not be seen with any accuracy in our preparations. The opening of the gullet, the rectum leading to the anus, and the opaque, dark intestines are all that can be positively established. Weidman in his description of *Pneumonyssus foxi* (*griffithi*) calls these tortuous structures lying just under the dorsum "intestines." Oudemans from a diagram of a larva, called them the "Malpighischen Gefässe." It is possible that we have mistaken his allusion. The eggs when present are just distinguished as bodies of oval outline lying eccentrically within the abdomen. Very large egg-larvae show dim lines as of embryonic legs.

The *larvae* are octagonal in body outline, are six-legged and in general repeat the anatomy of the adult. One pair of walking tarsi is missing. No genital aperture can be seen. The abdomen is markedly shortened. The intestinal tubes are very large and very prominent, obscuring any anal plate that may be present. The larvae show peculiarly well the structures of hypostome and epistome, characters which we infer to be contsant thru the molt into the adult stage, since we have used them for description of these parts after failure to clearly make them out in the adults.

A key given by Ewing in his "Notes on the Lung Mites of Primates" (1929), makes clear the relative position of the new species and we have adapted his key to include our new species.

1. Tarsal claws of second and third pairs of legs large, angulate, projecting laterally and more conspicuous than the pulvillus; abdomen of gravid females elongate and swollen; and, in some specimens separated from cephalothorax by transverse folds. Occurring in Ethiopian primates.

A—With two pairs of stigmata, abdomen in gravid females very long. Found in a guenon, *Cercopithecus schmidtii*
Pn. duttoni, Newstead and Todd

B—With but a single pair of stigmata; abdomen not so long in gravid females. Found in a baboon, *Papio sp.* . . . *Pn. congoensis,* Ewing

2. Tarsal claws of first leg pair large and well developed, conspicuously projecting at right angles from the tarsus tip, often recurving, without a joint piece in the tarsus tip. Palpi of five segments. Second, third and fourth leg pairs with slender pedicels, small claws and delicate pulvilli. No transverse fold between cephalothorax and abdomen. Occurring in South American primates and dogs. Dorsal plate present.

A—Sternal plate present. Chelicerae fused, forming one piece. Vulva inconspicuous. Occurring in a South African monkey, *Lagothrix* sp. . . . . *Pn. stammeri,* Vitzthum

B—Sternal plate absent. Chelicerae chelate. Vulva marked in its position and structure. Palpi with two terminal setae. Occurring in a dog, *Canis familiaris.* . . . . . *Pn. caninum,* n. sp.

3. Tarsal claws of second and third pairs of legs much reduced, not angulate, not projecting laterally and less conspicuous than the pulvillus; abdomen of gravid females swollen but not elongate and never separated from cephalothorax by a transverse fold. Occurring in Oriental primates.

A—Dorsal plate present; pulvilli constant on legs I, II, and III. Occurring in *Macacus rhesus* . . . . . *Pn. griffithi,* Newstead

B—Dorsal plate wanting; pulvilli poorly developed in some specimens. Occurring in a Javan monkey, *Cynocephalus* sp.

Pn. simicola, Banks

Halarachne species are distinctly different morphologically. A summary of the variances is valuable chiefly in its bearing upon the host relationships. *Halarachne* and *Pneumonyssus* species probably have had origin in the case of different free-living dermanyssid mites. Yet somewhat similar habitats, within the dog family CANIDAE, have not as yet been able to merge the parasite morphologic modifications to a closer resemblance. *Halarachne* species are grossly larger, elongate, with more setae; the palpi are relatively longer, and the sensory setae upon them have a split hair without a terminal spine; the chelicerae are modified to combs and spiny blades; all four pairs of legs are of the walking type. The peritremata and stigmal plates vary markedly in shape, in size, in associated tracheation; sternal plates and even genital plates are present, along with the anal plate common to both genera.

The species *Pneumonyssus stammeri* (Vitzthum, 1930), of a South American monkey, thru which lies the closest observed linkage, is so similar as to warrant a marked comparison. The leg structure, in general, is identical in that both the latter three pairs of legs own ambulacra, the first pair bearing a conspicuous set of gripping claws. In the tarsus tip of leg I, however, either a joint piece below the gripping hooks has not been observed or it is absent in the species *Pn. stammeri.* Likewise the joint pieces of the ambulacral hooks are neither observed nor drawn. In the new mite these structures are inescapable. The dorsal plate of *Pn. stammeri* is smaller, more leaflike, with more setae. There is a sternal plate, (missing in our species). The anal plate is duplicatory. The vulval slit in *Pneumonyssus stammeri* is anterior to the site observed in the dog mite here described, and much less prominent. Vitzthum calls it "einen kaum sichtbaren Querspalt zwischen den Coxae IV." The stigmata vary somewhat in shape and structure. The palpi in *Pneumonyssus stammeri* lack the outer shorter spine of the terminal segment. The chelicerae of *Pneumonyssus stammeri* are small, simplified to one piece (or the chelate structure has been missed in the few specimens available), but possess the same small spines on the exteriors of the fused mandibles that we have observed upon the movable outer members of the chelicerae of the new mite. The peritremata are probably very similar, judging from word description alone. In total the resemblance is striking. There is a slight difference in size and shape as well, but these could easily be accounted for if more constant features were not at odds. It is from these points of divergence and similarity, slight though they may be, that a new speices of mites is suggested. This from the dog host (*Canis familiaris*).

The bulldog host apparently showed no symptoms of the infection.

For some time previous to observation at the veterinary clinic of this school, the dog had been steadily losing flesh, the mouth had developed sores and the feces had had a very foul odor. The animal was brought to the clinic and for four days was under observation, during which time only the urinary tract symptoms of canine Bright's disease were noted, along with the systemic symptomatology that accompanies retention of urine wastes. Sneezing or other indications of nasal irritation were not specially noted if they were at all present. The post-mortem examination of the head and respiratory tract revealed necrosis of the oral mucosa, some congestion of the eyes, only a darkening of the mucosa of the frontal sinuses in which the mites were discovered, coupled with negative pleura, pericardium and lungs. The lungs were without tubercles; merely showed the anthracosis of a city animal. The kidneys both showed a firmly adherent capsule of irregular surface, pitted and contracted with scar tissue; on section the tissue was very firm, the cortex light-colored from extensive fibrotic changes; some small cysts were noted. The tubules of the medulla showed considerable dilatation. The urinary bladder was distended, filled with a light, cloudy urine, containing white mucoid material. The prostate gland showed marked enlargement and was soft in consistency. Here again several cysts were noted, some of these containing bloody fluid. In the center of the organ there was a large cavity containing fluid pus.

The simple darkening of the sinus mucosa is hardly indicative of serious pathological changes due to the mite infection. Yet so large a number of active parasites can scarcely be conceived to inhabit the animal's sinuses without producing at least a very considerable irritation, such as was observed in the *Pneumonyssus* infection described by Helwig (1925) in experimental monkeys. In his cases, six monkeys showed "peculiar pulmonary lesions at first mistaken for tuberculosis . . . from every lesion of which were recovered two or more arachnid parasites." The lesions in these cases suggest that closer observation may reveal greater evidence of damage or we may have somehow been misled in habitat assignation by mass migration. Additional cases must be studied to provide more accurate data upon the condition, since the surprise of the discovery lost many opportunities for observation. Very probably the infection of dogs is a rarity. In the autopsy records here, only once before was there a report of such mites. Autopsies since the discovery of the mite have been fruitless. At least the fact that an acarina related to the genus *Halarachne* may attack the dog, and possibly simultaneously his close taxonomic relatives the foxes and wolves, and may there localize in the frontal sinuses is of real interest. The fact of its undoubted close similarity to the bronchial passage mite of sea mammals and the lung mites of primates, its habitat, morphology and life cycle make the mite of more than ordinary significance. The apparent low pathogenicity of the

mites would correspond to the opinions of Weidman (1925). Helwig's conclusions and summary of acarine infections in man assists in overriding the possibility that it may have been an adaptation of free-living mites, or chance contact with monkeys or sea mammals; the case history of the dog also precludes these possibilities. The anatomy of the mite and its close relationship to *Halarachne* clinch the point. It is curious that of the human cases listed by Helwig, in his review of the literature, no less than six of the twelve infections—all proven as etiological,—dealt with the urino-genital tract. Most of them named "cyst formation" with inflammatory processes as the pathological picture. The cysts noted in this case, in spite of the dissimilarity of the tracts involved, may have some significance.

Dr. H. Graf Vitzthum, discussing the adaptations of mite forms especially the *Pneumonyssus* group, gives a key that is a complement to the preceding one of Ewing. In comparing *Pneumonyssus* species from South America and Asiatic monkeys he notes that the fundamental differences are these:

1. In neo-tropical monkeys, that is to say New World monkeys, the acarine parasites have maxillipalpi of normal length and segmentation; this is in marked contrast to the unmistakably shortened palpi with reduced number of segments of the Asiatic-host mites.

2. In the New World mites the pedicels have a normal division into lobes as in free-living LAELAPTIDAE, this in contrast to a fused pedicel.

3. Greatly shortened peritremes in the middle of a longish stigmal plate in the former, in contrast to so rudimentary a breathing structure that it can scarcely be called a peritreme at all.

If one stresses these characters particularly, one must say that the neo-tropical species is more closely related to HALARACHNINAE than are the oriental species. For they agree particularly well with those of the *Halarachne*. The one African form, Ewing (1928) perhaps falls somewhere midway between the groups above compared.

From characters of the new mite, it may follow that its position in the classification is a step toward the *Halarachne* species. This would also follow from the variance in hosts, for the canine is but a first cousin to the seal, and phylogenetically considerably farther removed from the primates.

An interesting observation that has been the result of this study is possibility of a parturition by bursting, a natural Caesarean section that perhaps is universal in this group of acarina. At least we have it on authority of H. Graf Vitzthum that "No one has ever seen an oviposition (Eigellege)." Photomicrograph, Fig. 17, illustrates the condition. Drawings of the mites show uniformly, very large larvae within the gravid females, with the same disparity in size of the vulval slit. Vitzthum, German acarologist, gives this interesting life cycle of air passage

mites in general; "if one lumps the observations of the authors he gets as a picture the developmental cycle of such lung parasites. The egg stage is passed intrauterinely, the animals come into the world viviparously, skip any "nymph" stage and molt from the "larva" immediately into the adult, all of which could possibly but improbably indicate a parthenogenetic reproduction. (Weidman has described a male and all writers upon *Halarachne* species describe the male.) So far this is based only on supposition, but there is an extremely great probability for it, since a shortened sequence of developmental stages is proved. It would be surprising indeed if it were different for the parasites in the lungs of monkeys."

Much of the above is clearly inductive, and is the result of comparison and logic. It may be that we have accidentally added to the observed facts of the life cycle, for from the mites here described it is plain that gravid females show the embryos filling a large part of the abdomen, forcing the abdominal viscera to one side. These embryos at maturity are very considerably wider than the clearly seen vulva. It is easily conceivable that at the time of birth the vulval orifice stretches greatly to allow passage of the embryo, these being born viviparously, as Vitzthum supposes. The clearly defined labial bands would infer use of the vulva in birth as well as copulation. However, the marked tendency toward abdominal rupture is undeniable. Weidman has noted this also. The mere placing of the coverslips has been enough to break a large percentage of the gravid females. Death agonies may be responsible, or undue pressure in mounting, for this phenomenon illustrated in photomicrograph, Fig. 17. It is true that nowhere in mite life cycles is there any comparable condition. The closest that we can come phylogenetically to see a similarity is in the monogenetic flukes. That is a long departure, but it is a point to be decided by more experienced acarologists.

There are many points in the above description that are, in the final analysis, just short of guesswork. Or let us call them deductions from flattened specimens. They wait for their real elucidation upon section and dissection, rather than upon microscopy. The U. S. Nat. Mus. has assigned to the new species type No. 1364.

In addition to those who brought this mite to our attention we are indebted to Miss Lisa Neu of the Department of Bacteriology for her accurate translations of German publications.

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Explanation of Plates

Plate I

The line drawings are freehand by the authors, from cleared specimens, drawn as if transparent.

Fig. 1. Dorsal view of gravid female, showing dorsal plate, intestines (int.) and general conformation. Body length, 1.5 mm.

Fig. 2. Ventral view of gravid female, showing head parts, legs, peritremata (per.), general aperture, anal plate (a.p.) and mature embryo (m.em.). Body length 1.15 mm.

Fig. 3. Detail of tarsus with ambulacrum (amb.), showing hooks (h), joint pieces (j.p.) and caruncle (car.) typical of tarsi II, III, IV. Length of tarsus and pedicel entire 0.3 mm.

Fig. 4. Peritreme (per.), with spiracle opening (sp.), stigmal plate (st.p.) and beginning of trachea (tr.). Stigmal plate 0.22 mm.

Fig. 5. Anal plate, with anus and setae.

Fig. 6. Larval mite, hexapod and octagonal. Length 0.68 mm.

Fig. 7. Primary tarsus, with gripping claws, rocker socketpiece (so.p.) and sensory setae. Tarsus length 0.16 mm.

Fig. 8. Female genital slit, width 0.2 mm, vulval lips (v.l.) and corner articulations (c.a.).

Fig. 9. Chelicerae, with blunt fixed arm (f.a.) and movable spined chela (s.c.).

Fig. 10. Head parts; palpi (pa.); capitulum (cap.); chelicerae (ch.); hypostome (hyp.), terminal sensory setae, and gullet groove (g.g.) palpi 75–80 μ in length.

Plate II

Fig. 11. Anal plate, ventral view, showing triad of setae and irregular anal opening, button-like in stained specimens. Anterior to posterior 138 μ.

Fig. 12. Anterior dorsum of gravid female, with dorsal plate, leg pairs and head parts distinguishable. Mite had been crushed posteriorly, producing a false impression of girth.

Fig. 13. Vulva, ventral view, showing labial bands, posterior articulation shoulders, and edges of IV coxae. Width, shoulder to shoulder, 0.2 mm.

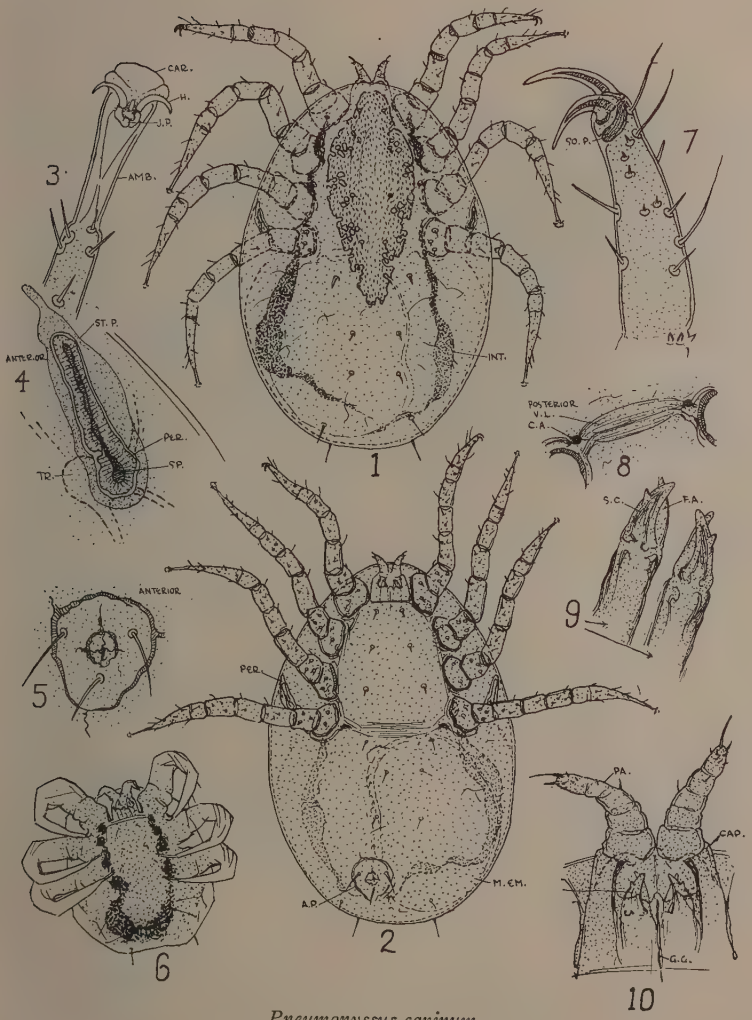
Fig. 14. Tarsus tips, showing end segments of the four tarsi. Claws and basal piece of tarsus I, the pedicel with caruncles, with hooks and joint pieces of tarsi II and III are clearly seen. Note setae and basal papillae. Terminal segments 198 μ, pedicel 54 μ.

Fig. 15. Chelicerae and palpi, ventral view. The chelate chelae are seen, but tiny spine on exterior edge of outer chela member is not in focus, nor is the entire matching chelicera. One palpus is dimly seen, with its short sensory wedge setae and terminal spine. Palpus 80 μ length.

Fig. 16. Larval mite, hexapod and showing faintly the octagonal shape. The intestines and head parts are defined. Length 0.6 mm.

Fig. 17. Gravid female, dorsal view with fully developed larval mite apparently in process of parturition. Note width of larva as compared to width between coxae IV. See vulva also. Total length of mite, 1.3 mm.

Fig. 18. Peritreme, with spiracle opening and just discernible stigmal plate. Note faint fluting of peritremal inner margin. One tracheole is seen. Length of peritreme 0.16 mm.



Pneumonyssus caninum

Pneumonyssus caninum

A NOTE ON THE PROBLEM OF ACQUIRED IMMUNITY TO ARGASID TICKS

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It has been shown (Trager, 1939a) that guinea pigs once infested with the ixodid tick, *Dermacentor variabilis* Say, develop an acquired immunity which effectively prevents subsequent groups of larvae from engorging. The immunity is a generalized one and lasts about 3 months. A similar acquired immunity is developed by rabbits and deer mice against the larvae of *D. variabilis,* by guinea pigs against the larvae of *D. andersoni,* and by rabbits against the larvae of *Haemaphysalis leporis-palustris. D. variabilis, D. andersoni* and *H. leporis-palustris* are all members of the family IXODIDAE. The ticks of this family have 3 stages in their life cycle and take one large blood meal in each stage, remaining attached to the host at each meal for a time varying from several days to about 2 weeks.

The majority of blood sucking arthropods, unlike the ixodid ticks, engorge in a relatively short time, and it was of interest to see whether an animal could develop any immunity to parasites of such a type. Ticks of the family ARGASIDAE, which in most of their stages engorge in a few minutes to a few hours, were selected for the work.

MATERIALS AND METHODS

Large numbers of *Argas persicus* Oken. were collected from chicken coops in the public market of Panamá. In order to obtain eggs and larvae engorged adults were kept in pairs of 1 ♂ and 1 ♀ in small vials held over moist sand. Groups of larvae which were to be compared in any one experiment were always counted out from the same vial and were hence of identical parentage. Unfed nymphs and adults were kept in large groups in vials over moist sand and counted numbers were removed when needed for experimental purposes. White Leghorn chickens from 6 weeks to 3 months old, obtained from a battery brooder and not previously exposed to ticks, were used for all the experiments with *A. persicus.* In a few experiments with nymphs and adults, the chicken was placed in a large covered battery jar equipped with paper toweling on the

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bottom and a raised wooden platform. This simulated natural conditions, since the ticks could hide under the platform during the day and feed on the chicken at night. In most of the work, the pill box method previously described was used, one 1¼″ pill box being attached to either side of a chicken on the region underneath the wing, a portion of skin which in young chickens is almost free from feathers. In such a covered box the majority of unfed nymphs and adults would engorge within 10 minutes, but they were usually left overnight to give as many as possible an opportunity to feed. The larvae, as is normal for the species, engorged within 5 to 8 days.

Ornithodoros venezuelensis Brumpt was collected from native houses in several villages in the interior of the Republic of Panamá. Eggs and larvae were secured by keeping several males and females together in small vials. In this way, one vial would contain enough larvae for a single experiment. Guinea pigs equipped with a 1½″ pill box on each side were used. The larvae, as well as the nymphs and adults of *O. venezuelensis,* engorged within an hour or less after attaching, but they were usually left on the host several hours or overnight so that as many as possible might feed.

TABLE 1.—*Acquired immunity to the larvae of* Argas persicus

Exper. No.	Chick No.	Previous treatment	Ticks put on			No. engorged
			No.	Date	Side	
				1939		
1	702	Fed upon by 118 nymphs and adults from 6/22 to 6/30/39 not restricted to 1 region of body	50	7/12	Left	21
	703	Fed upon by 7 larvae 7/6 to 7/10 on right side	50	7/12	"	38
	704	Fed upon by 100 nymphs and adults from 6/23 to 6/30 not restricted to 1 region of body and by 24 on right side only on 7/6	50	7/12	"	26
2a	720	Fed upon by 203 nymphs and adults on right side only from 7/8 to 7/17	100	7/22	"	43
	722	Fed upon by 202 nymphs and adults on right side only from 7/6 to 7/17	100	7/22	"	33
	724	Wore pill box on right side 7/8 to 7/17..	100	7/22	"	72
2b	721	Fed upon by 113 nymphs and adults on right side only from 7/8 to 7/12	100	7/20	"	49
	723	Fed upon by 103 nymphs and adults on right side only from 7/8 to 7/12	100	7/20	"	29
	725	Wore pill box on right side 7/8 to 7/12...	100	7/20	"	64
3	704	See Exper. 1	100	7/31	"	14
	726	42 larvae engorged on 7/22 to 7/29	100	7/31	"	36
4	716	Fed upon by 92 nymphs and adults from 7/6 to 7/13 not restricted to 1 region of body	100	7/20	Right	45
	717	Fed upon by 187 nymphs and adults from 7/6 to 7/13 not restricted to 1 region of body	100	7/20	"	56
	718	Fed upon by 11 nymphs from 7/11 to 7/13	100	7/20	"	4*
	719	Not exposed to ticks	100	7/20	"	50
5	717	See Exper. 4	100	7/31	"	2
	718	See Exper. 4	100	7/31	"	45
	719	See Exper. 4	100	7/31	"	33

* Chicken 718 scratched its box loose soon after the larvae were put in, so that most of them escaped.

RESULTS

I. *Argas persicus.* The results with nymphs and adults need not be given in detail. As many nymphs or adults would engorge upon a chicken previously fed upon by 100 to 150 nymphs over a period of 1 to 2 weeks as on a chicken not previously exposed. This was true even if successive batches of 30 to 40 ticks were placed repeatedly over the same region of the body. Usually 80 to 100 per cent of the ticks became engorged.

One infestation with larvae (about 50 engorged out of 100 applied to each chicken) did not confer any immunity to a second infestation with larvae. However, repeated infestations with a large number of nymphs and adults appeared to confer a slight immunity to the larvae as shown in Table 1, Exper. 1, 2, and 3. In another experiment (4), somewhat similar to these, no immunity was developed, but when chickens 717, 718, and 719 were again exposed to larvae much fewer engorged on 717, previously fed upon by 187 nymphs and 56 larvae, than on 718, previously fed upon by 11 nymphs and only 4 larvae, or 719, previously fed upon by 50 larvae only (Exper. 5). As might have been expected from these results, the intracutaneous or intraperitoneal inoculation of extracts of *Argas persicus* did not produce any immunity to larvae, nymphs or adults.

Attempts to demonstrate complement fixation (see Trager, 1939b) with serum from the partially immune chickens as antibody and extract of *Argas persicus* as antigen failed because of the very high anti-complementary activity of the two antigens tried. One, a saline extract of nymphal and adult ticks, was anti-complementary even at a dilution of 1 : 25,000 on the basis of the weight of ticks used. The other, an extract of larval ticks, lost its anti-complementary effect at a dilution of 1 : 20,000 on a weight basis. At this dilution it did not fix complement in the presence of immune chicken serum.

II. *Ornithodoros venezuelensis.* In a small series of suitably controlled experiments, there was no evidence of any fewer larvae feeding on guinea pigs previously exposed to even as many as 5 successive infestations, each with about 50 ticks, over a period of 2 to 3 weeks than on animals not previously infested. Definite red marks were noted in the skin at the site of attachment of larvae as well as nymphs and adults, but these did not appear any different after a fifth or sixth infestation than after a first.

DISCUSSION

It is evident that the exposure, over a relatively short time, of chickens to infestation with *A. persicus* or of guinea pigs to infestation with *O. venezuelensis* does not result in any immunity which is capable of acting within the 10 minutes to an hour required for engorgement by the nymphs and adults of the former tick and by all stages of the latter.

That the nymphs and adults of *A. persicus* do, however, inject their host with antigenic substance is demonstrated by the partial immunity which repeatedly infested chickens exhibit against the larvae of the fowl tick. One may assume that, during the several days required for the larvae to engorge, a cellular reaction, of the type described in the studies with *Dermacentor variabilis,* occurs in the immune animals.

In spite of the negative results of the present study, the possibility remains that a very prolonged exposure of an animal to repeated infestations with a rapidly feeding blood-sucking arthropod might eventually result in an immune response which could occur within the short time of attachment of the parasite. This possibility is strengthened by the indications that the rapidly feeding nymphs and adults of *A. persicus* do apparently inject effective amounts of an antigenic substance.

Cherney, Wheeler and Reed (1939) have recently reported a partial "immunization" of people to flea bites. From their work it is impossible to decide whether the "immune" people were immune to the fleas themselves or to the effects of the flea bite. This is an important and necessary distinction and has been previously emphasized (Trager, 1939a). A person who did not react to flea bites could be repeatedly bitten without his being aware of it. One can cite again the observation of Rozeboom (1936) that the donor of blood to a colony of *Anopheles albimanus* soon became immune to the effects of the bites, although the mosquitoes continued to feed on him as well as ever. An experimental demonstration of a true immunity against rapidly feeding blood-sucking arthropods themselves, that is, an immune mechanism which would actually prevent them from feeding, would be most interesting and important.

SUMMARY

Chickens which had been repeatedly infested with nymphs and adults of *Argas persicus* did not show any immunity to these stages of the tick, which engorge in 5 to 10 minutes. The chickens did, however, exhibit a partial immunity to the larvae of *Argas persicus,* which require at least 4 days for engorgement.

Guinea pigs exposed to repeated infestation with *Ornithodoros venezuelensis,* all stages of which engorge rapidly, showed no immunity to any of the stages of this tick.

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A RECONSIDERATION OF THE ACANTHOCEPHALAN FAMILY RHADINORHYNCHIDAE*

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The family name RHADINORHYNCHIDAE (PALAEACANTHOCEPHALA) dates from 1923 when Travassos elevated to family rank the sub-family RHADINORHYNCHINAE proposed by Lühe in 1912 to include the genus *Rhadinorhynchus* and the concept of the genus *Serrasentis,* then known as *Echinogaster.* In elevating the rank of the group, Travassos added the genus *Polyacanthorhynchus* to the new family concept.

Travassos (1926) in his diagnosis of the RHADINORHYNCHIDAE emphasized the extreme elongation of the proboscis and the presence of eight elongated cement glands as distinctive for the family. Chandler (1934) called attention to the fact that Lühe (1911) and other workers had not mentioned the number of cement glands in *R. pristis* and in some other species of *Rhadinorhynchus,* but stated "it is probable that if the type species *R. pristis* had less than the usual eight, the fact would have been mentioned by Lühe."

During the past quarter century numerous studies have extended the original concept of the genus *Rhadinorhynchus* as well as the concept of the family, and have added several distinct genera to the family group.

Meyer (1932) in his monograph on the ACANTHOCEPHALA assembled nine genera under this family caption. To these, still other more recently recognized genera must be added, until at present thirteen genera are attributed to this family (See Table 1). Among the diverse species now included in the expanded concept of the family the form of the proboscis and number of cement glands, which Travassos considered as diagnostic, now fail as unifying characters on which to base the family. Several of the forms have superficial resemblances to the genotype, but differ in essential morphological details. Chandler (1934) recognized two new genera (*Gorgorhynchus* and *Nipporhynchus*) chiefly on the basis that each has four cement glands in the male instead of the eight characteristic for *Rhadinorhynchus.* In order to evaluate the various discrepancies which have been insinuated into the family concept, it has seemed timely to offer a critical reconsideration and rearrangement of the genera and species ascribed to the RHADINORHYNCHIDAE.

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The current concept of the family is that given by Meyer (1932): "Von leptosomen Körperbau mit keulenförmiger langer Proboscis, meist schlauchformigen Zementdrüsen und spidelförmigen Eiern mit polarer Ausbuchtung der Hüllen: Rumpf vorn ganz oder teilweise bestachelt oder Bestachelung sekundär fehlend; Haut entweder mit wenigen Riesenkernen oder mit zahlreichen kleinen Kernfragmenten."

Conspicuous variations from the current concept of this group are concerned with the proboscis and the cement glands. In the type genus the proboscis is clavate and relatively long and slender. In several genera, namely, *Cleaveius, Serrasentis, Micracanthocephalus, Gorgorhynchus,* and *Aspersentis,* there is significant departure from the type. In *Cleaveius* the proboscis is exceedingly short and cylindrical. Similarly in *Micracanthocephalus, Gorgorhynchus,* and *Aspersentis,* it is relatively short and cylindrical, while in *Serrasentis* it is nearly globular.

An analysis of all the available characters has demonstrated that the cement glands of the male provide a basis for aligning the genera here considered into two natural groups, as shown in Table 1.

TABLE 1.—*The genera of the Rhadinorhynchidae grouped according to the number of cement glands*

Eight cement glands	Four cement glands
Illiosentis Van Cleave and Lincicome (in press)	*Aspersentis* Van Cleave, 1929
Leptorhynchoides Kostylev, 1924	*Cleaveius* Subramanian, 1927
Polyacanthorhynchus Travassos, 1926	*Filisoma* Van Cleave, 1928
Rhadinorhynchus Lühe, 1911	*Gorgorhynchus* Chandler, 1934
Tegorhynchus Van Cleave, 1920	*Micracanthocephalus* Harada, 1938
Telosentis Van Cleave, 1923	*Nipporhynchus* Chandler, 1934
	Serrasentis Van Cleave, 1923

Those genera having eight cement glands bear a club-shaped, much elongated proboscis, and conversely, all but two of the genera possessing four cement glands bear a relatively short proboscis. In *Nipporhynchus* and *Filisoma* there are but four cement glands, but the proboscis is of relative proportions characteristic for the genus *Rhadinorhynchus.*

To give recognition to the two profoundly different conditions in the cement glands among the genera currently ascribed to the family RHADINORHYNCHIDAE, the present writers hereby propose to restrict the family RHADINORHYNCHIDAE to those genera having eight cement glands. For the remaining genera, characterized by the possession of four cement glands, a new family is proposed under the name GORGORHYNCHIDAE with *Gorgorhynchus* Chandler, 1934 as type, and in addition including *Aspersentis, Cleaveius, Filisoma, Micracanthocephalus, Nipporhynchus* and *Serrasentis.* The following definition is proposed:

GORGORHYNCHIDAE n. fam.

Palaeacanthocephala of diverse range in body length. Proboscis clavate or cylindrical in form, sometimes globular, and usually short. Proboscis hooks may show dorso-ventral differentiation. Proboscis sheath double-walled. Anterior

spination on body may be in single or double fields or wanting. Posterior spination absent. Lemnisci about as long as proboscis sheath, usually much longer. Cement glands four in number, either very long and tubular or short and pyriform. Type genus *Gorgorhynchus* Chandler, 1934.

The position of the genus *Aspersentis* is only tentative, since the exact number of cement glands which it possesses has not been definitely established. Though the genus exhibits a change in form of the proboscis it may be regarded for the present as a member of the GORGORHYNCHIDAE.

Meyer (1932: 60) includes the genus *Filisoma* Van Cleave, 1928 in the family RHADINORHYNCHIDAE. In the original material on which *Filisoma indicum* is based, the condition of the cement glands could not be determined with certainty. In a recent paper, Harada (1938) has described a new species, *Filisoma microcanthi,* but since he had no males this additional reference to the genus gives no support to its family relationships. The senior author of this paper has in his collection specimens of an undescribed species of *Filisoma* in which four very much elongated cement glands are observable. Since this condition is in agreement with those set up as distinguishing the GORGORHYNCHIDAE, the present writers propose the assignment of *Filisoma* to the family GORGORHYNCHIDAE.

The following key shows how the genera of the GORGORHYNCHIDAE may be differentiated.

KEY TO THE GENERA OF THE GORGORHYNCHIDAE

1. a) Body with ventral pseudosegmental cuticular combs or almost fused rows of spines ..... *Serrasentis.*
   b) Body without pseudosegmental cuticular combs or rows of spines ..... 2.
2. a) Body spination present ..... 3.
   b) Body spination lacking ..... 7.
3. a) Cement glands extremely long, tubular, occupying a considerable portion of the body ..... 4.
   b) Cement glands short and pyriform ..... 5.
4. a) Prominent arcuate hooks present at base of proboscis ..... *Nipporhynchus.*
   b) Prominent arcuate hooks absent at base of proboscis ..... *Gorgorhynchus.*
5. a) Proboscis hooks show profound dorso-ventral asymmetry—hooks of ventral surface more than twice the size of those on dorsal surface .... *Aspersentis.*
   b) Proboscis hooks exhibit no apparent dorso-ventral differentiation ..... 6.
6. a) Proboscis hooks arranged in 18 longitudinal rows of 4 hooks each *Cleaveius.*
   b) Proboscis hooks arranged in 12 longitudinal rows of 8 or 9 hooks each *Micracanthocephalus.*
7. a) Brain at posterior tip of receptacle ..... *Filisoma.*
   b) Brain anterior in receptacle ..... *Nipporhynchus* (in part).

The following definition of the family RHADINORHYNCHIDAE, as here restricted, is proposed:

RHADINORHYNCHIDAE Travassos, 1923, emend.

PALAEACANTHOCEPHALA of considerable length. Proboscis very long, clavate. Proboscis sheath double-walled. Proboscis hooks show dorso-ventral differentiation. Anterior spination on body present or may be secondarily lost. Posterior spination may be present. Lemnisci about as long as proboscis sheath but may be longer. Cement glands eight in number, usually short and pyriform or may be elongated. Type genus *Rhadinorhynchus* Lühe, 1911.

The following is a key to the genera of the family RHADINORHYNCHIDAE:

1. a) Body spination lacking ..... *Leptorhynchoides.*
   b) Body spination at least in members of one sex ..... 2.
2. a) Spines around posterior extremity at least in members of one sex ..... 3.
   b) Body spines lacking around posterior extremity ..... 4.
3. a) Form of posterior extremity not conspicuously modified. Scattered spines around posterior end of both sexes ..... *Telosentis.*
   b) Posterior extremity of female modified as dorsal and ventral protuberances of which the dorsal bears the genital opening. Spines around genital extremity of female only ..... *Illiosentis.*
4. a) Proboscis enveloped in a thick, hyaline membrane ..... *Tegorhynchus.*
   b) Proboscis not enveloped by a conspicuous hyaline membrane. Body spination restricted to one or two scattered fields at anterior extremity ..... 5.
5. a) Proboscis bearing about forty longitudinal rows of hooks on anterior end and smaller number at base ..... *Polyacanthorhynchus.*
   b) Proboscis bearing not more than twenty-six longitudinal rows of hooks *Rhadinorhynchus.*

Through an error in transcription, the senior author of this article (Van Cleave, 1924) attributed *Echinorhynchus socialis* Leidy, 1851 as type of the genus *Serrasentis.* Linton (1932) called attention to this error while Meyer's monograph (1932) was in press, hence Meyer perpetuated the error of citing *S. socialis* as type of *Serrasentis.* Linton (1932) is correct in his position that *Echinorhynchus socialis* is a synonym of *Echinorhynchus gadi* Müller, while *Echinorhynchus sagittifer* Linton, 1889, cited in error as a synonym of *Serrasentis socialis,* is a valid species. So far as is known *Serrasentis sagittifer* is the only representative of this genus found in North American hosts.

Harada (1935) applied the preoccupied name *Micracanthorhynchus* to a generic concept based on a previously undescribed species, *M. motomurai.* In 1938, Harada renamed this genus *Micracanthocephalus.* According to the description of the genotype, the genus is clearly a member of the family GORGORHYNCHIDAE. In the paper just cited, Harada (1938: 419) describes another new species, *M. dakusuiensis,* which, though he observed six cement glands, he considers as congeneric with *M. motomurai.* However, in his description of the genotype he recorded four cement glands, which is in full agreement with the concept of the family GORGORHYNCHIDAE. The present writers in reviewing the GORGORHYNCHIDAE and the RHADINORHYNCHIDAE have found in each family complete agreement in the number of cement glands present in

the representatives of all the genera. Consequently we question either the generic assignment of *M. dakusuiensis,* or the correctness of Harada's observation that this species has six cement glands.

Harada (1935) described *Rhadinorhynchus trachuri* which possesses but four cement glands and is therefore not congeneric with other species of *Rhadinorhynchus.* In all essential features it agrees with the concept of *Nipporhynchus* and is therefore assigned to this genus. In 1938, Harada described *Rhadinorhynchus nudus* which, though lacking body spines, agrees fully with the concept of *Nipporhynchus* and is also assigned to this genus.

Chandler (1934) in recognizing the genus *Nipporhynchus* gave only a provisional definition based on the specific definition of *Rhadinorhynchus katsuwonis* by Harada (1928). As shown later in this paper, *Nipporhynchus katsuwonis* is a direct synonym of *Nipporhynchus ornatus.* Because of the fact that many details in the morphology of the latter species were unknown, Chandler tentatively ascribed it to the genus *Nipporhynchus.* Males of this species, previously unknown, have been observed by the senior author in a collection taken by the Allan Hancock Pacific Expedition from *Gymnosarda* of Charles Island in the Galapagos group. The presence of four cement glands confirms Chandler's tentative assignment of *ornatus* to the genus *Nipporhynchus.*

With the addition of several species to this genus, it becomes possible to shear away specific characters and to re-characterize the genus *Nipporhynchus* in terms which reflect essential generic features.

Nipporhynchus Chandler, 1934, emend.

GORGORHYNCHIDAE with long proboscis, armed with many rows of hooks which are longer and stouter on ventral surface than on dorsal. Basal row of hooks forming a complete circle without differentiated ventral crescent. Proboscis sheath double-walled. Anterior region of body usually beset with spines which may be in single or double fields. Lemnisci ribbon-shaped, about as long as proboscis sheath. Testes elongated, cylindrical, or club-shaped and may be paired.

By original designation Chandler made *Rhadinorhynchus katsuwonis* Harada, 1928, type of the genus *Nipporhynchus.* In a series of unpublished observations, the senior author of this paper has determined that *N. katsuwonis* is a direct synonym of *Nipporhynchus ornatus* (Van Cleave, 1918). Therefore, *Nipporhynchus ornatus* (Van Cleave, 1918) becomes genotype.

The genus *Heterosentis,* based on *Echinorhynchus heteracanthus* of von Linstow (1896) by the senior author of this paper in 1931, was then assigned to the RHADINORHYNCHIDAE. It now seems probable that this generic assignment was incorrect. Meyer (1931) transferred the genus *Heterosentis* to the family QUADRIGYRIDAE. This assignment is wholly untenable. The lack of giant nuclei in the subcuticula and the presence

of six separate, pyriform cement glands in *Heterosentis* are characters wholly incompatible with the QUADRIGYRIDAE as well as with the order EOACANTHOCEPHALA to which it pertains. *Heterosentis* is clearly a member of the order PALAEACANTHOCEPHALA. Yamaguti (1935: 273) has assigned *Heterosentis* to his family ARHYTHMACANTHIDAE and while the present writers are not certain as to the validity of this family they tentatively accept the assignment withholding *Heterosentis* from the RHADINORHYNCHIDAE and GORGORHYNCHIDAE discussed in the present paper.

SUMMARY

The family RHADINORHYNCHIDAE Travassos, 1923, as conceived by Meyer (1932) and emended by Chandler (1934) contains two distinct groups of genera sharply separable on basis of the number of cement glands and in part supported by form of the proboscis. A new family GORGORHYNCHIDAE is proposed and diagnosed for those genera of the older concept of the RHADINORHYNCHIDAE possessing four cement glands, with *Gorgorhynchus* Chandler, 1934, as genotype. Additional genera are assigned to the GORGORHYNCHIDAE as follows: *Aspersentis, Cleaveius, Filisoma, Micracanthocephalus, Nipporhynchus,* and *Serrasentis.*

A restricted definition of the RHADINORHYNCHIDAE is given. In addition to *Rhadinorhynchus,* the genotype, this family includes the following genera: *Illiosentis, Leptorhynchoides, Polyacanthorhynchus, Tegorhynchus,* and *Telosentis.*

Nipporhynchus katsuwonis (Harada, 1928) is considered as a direct synonym of *Nipporhynchus ornatus* (Van Cleave, 1918), hence the latter becomes genotype. An emended definition of *Nipporhynchus* is presented. *Rhadinorhynchus trachuri* (Harada, 1935) and *Rhadinorhynchus nudus* Harada, 1938, are ascribed to the genus *Nipporhynchus.*

Keys to the genera of GORGORHYNCHIDAE and RHADINORHYNCHIDAE are presented.

Heterosentis (Van Cleave, 1931) originally assigned to the RHADINORHYNCHIDAE, cannot be included in the QUADRIGYRIDAE as proposed by Meyer (1931). Assignment to the family ARHYTHMACANTHIDAE as proposed by Yamaguti (1935) is tentatively accepted.

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A NEW TREMATODE, *MYOTITREMA ASYMMETRICA*, N. G., N. SP., (LECITHODENDRIIDAE) FROM THE LITTLE BROWN BAT

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Among the parasites of 16 little brown bats (*Myotis lucifugus*) found in an abandoned house on the shore of Lake Minnetonka, near Wyzata, Minnesota, were two specimens of an undescribed trematode. A description of the new genus and species follows.

Myotitrema asymmetrica, n. g., n. sp.

Specific diagnosis: LECITHODENDRIIDAE : PLEUROGENETINAE :—Body elongate, 0.77 to 1.15 mm long by 0.65 to 0.70 mm wide, flattened dorso-ventrally. Prominent spines covering two-thirds of the body surface. Oral sucker subterminal, 0.07 to 0.08 mm long by 0.10 to 0.11 mm wide. Pharynx 0.04 mm long by 0.03 to 0.05 mm wide. Esophagus relatively long. Intestinal fork situated at anterior margin of acetabulum. Intestinal cecae moderately long, reaching past the body middle. Acetabulum somewhat larger than oral sucker, 0.11 to 0.13 mm in diameter, situated anterior to the middle of the body. Testes subtriangular to ovate, average diameter 0.13 to 0.14 mm, postacetabular, and located just anterior to the middle of the body. Seminal vesicle sinuous, lying free in the body parenchyma, maximum width 0.037 to 0.038 mm. Prostate cells numerous but diffusely arranged. Genital pore on left body margin at the level of the acetabulum. Ovary subtriangular with a tendency toward slight lobing, situated immediately anterior to the testes and on the right side of the body. Neither seminal receptacle nor Laurer's canal could be observed. Vitellaria consisting of elongate follicles, characteristically diffuse, extending from the level of the acetabulum to the region just posterior to the pharynx. Uterus largely filling posterior half of body, terminating in swollen transverse sling which extends from level of ovary across body to genital pore. Eggs similar to those of related genera, 19 × 12 microns.

Host: Myotis lucifugus (LeConte).

Location: Intestine.

Locality: Lake Minnetonka, Hennepin County, Minnesota.

Specimens: Type, U. S. Nat. Mus.; paratype in author's collection.

Myotitrema

Generic diagnosis.—Genital pore sinistral, on the body margin at the level of the acetabulum. Cirrus sac absent. Seminal vesicle coiled, long and slender. Testes opposite, near the body middle. Ovary immediately in front of the right testis. Uterus extensive, filling posterior half of body. Suckers subequal. Intestinal cecae reaching as far as the middle of the body. Excretory bladder "V"-shaped.

Type species: Myotitrema asymmetrica.

In his valuable discussion of the taxonomy of the family LECITHODENDRIIDAE, Mehra, 1935, includes the following subfamilies in that group: ANCHITREMINAE Mehra, 1935; LECITHODENDRIINAE Looss, 1902; PLEUROGENETINAE Looss, 1899; PHANEROPSOLINAE Mehra, 1935; EXOTIDENDRIINAE Mehra, 1935; EUMEGACETINAE Mehra, 1935.

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To this list may be added GYRABASCINAE Macy, 1935, in which the genital pore is postacetabular, lateral to nearly median, and seminal vesicle sinuous, lying free in the body parenchyma. Mehra places *Ganeo* Klein, 1905, in the subfamily PLEUROGENETINAE, although it does lack a definite cirrus sac. *Myotitrema* also appears to belong to the PLEUROGENETINAE, but is not close to *Ganeo* since the sex organs have a markedly different arrangement. In *Myotitrema* the genital pore is lateral to the acetabu-

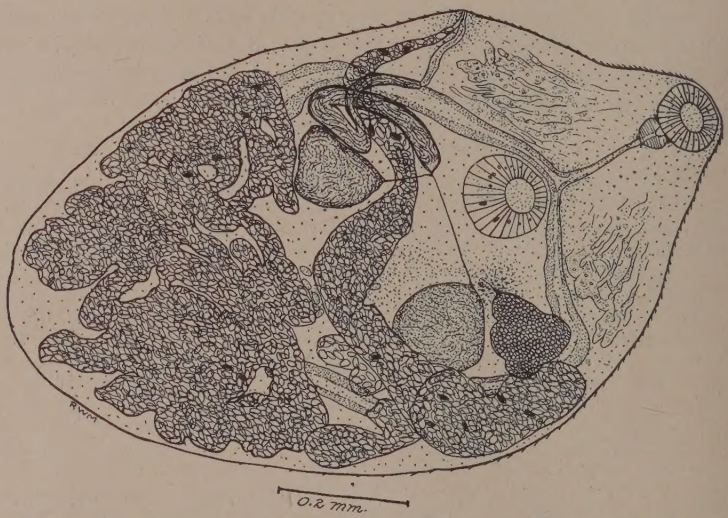

FIG. 1. *Myotitrema asymmetrica*, type, dorsal aspect. Drawn with aid of the camera lucida.

lum; in *Ganeo* it is anterior to the acetabulum. *Myotitrema* seems to be a specialized offshoot of *Pleurogenes* Looss, 1896, due to the fact that the genital pore is sinistral on the body margin, the ovary is pretesticular, the vitellaria are in the anterior part of the body, and the cecae are rather long. The chief distinguishing character is the coiled seminal vesicle lying free in the parenchyma.

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RESEARCH NOTES

NOTE ON CULTIVATION OF *TRICHOMONAS FOETUS*

Trichomonas foetus can be maintained for an apparently indefinite period in a culture medium which contains no thermolabile substance. Such a medium has been used in this laboratory since May 13, 1939. The cultures, which are bacteria-free, have been transferred at approximately weekly intervals.

The medium used is that of Rees (1937, Am. J. Hyg. **26**: 2) except that after addition of serum the entire mixture is autoclaved at 15–18 pounds pressure for 15 to 20 minutes. The medium may be sterilized for 40 minutes or for 15 to 20 minutes on three successive days without any harmful effects.

Blood serum of the horse, cow or sheep may be used with equally satisfactory results. The amount of serum used may be varied from one-half per cent to four per cent with little effect on longevity of cultures. If a series of cultures is considered as a unit the above holds. However, individual tubes will be found showing a considerably increased length of life associated with the larger amounts of serum. Autoclaving the serum does not decrease longevity below that of cultures grown with unautoclaved serum.

One objection to this method of cultivation is that a flocculent precipitate forms after several days standing and this may interfere with some types of experimental work. Also the initial rate of division and the maximum number of trichomonads per cubic centimeter is some times less than in media containing unautoclaved serum.

The autoclaved medium would seem to offer a means of long continued bacteria-free cultivation under conditions where it is difficult to obtain sterile sera or where filtration facilities are inadequate. The danger of bacterial contamination is also reduced since the sterile tubes do not have to be opened for the introduction of serum and then again for inoculation. The act of introducing the trichomonads is the only procedure which is liable to introduce a contaminant.

Long continued cultivation by the method herein described demonstrates the fact that at least one pathogenic flagellate does not require a thermolabile substance for the maintenance of normal metabolism.—GEORGE E. DANIEL, *Live Stock Sanitary Service Laboratory, University of Maryland.*

DEVELOPMENT OF *DIPLOSTOMUM FLEXICAUDUM* (CORT AND BROOKS) IN THE CHICKEN BY FEEDING PRECOCIOUS METACERCARIAE OBTAINED FROM THE SNAIL INTERMEDIATE HOST

Cort and Brackett (1937, J. Parasitol. **23**: 545–546) reported finding diplostomula in specimens of the beach snail, *Stagnicola emarginata angulata* (Sowerby), during routine autopsies of these snails collected in the Douglas Lake Region, Michigan. They concluded that these strigeid metacercariae were precociously developed diplostomula of *Diplostomum flexicaudum* (Cort and Brooks) whose life cycle was worked out by Van Haitsma (1930, Pap. Mich. Acad. Sc., Arts and Letters **13**: 483–516). This author showed that the cercariae of this species penetrate the common sucker, *Catostomus commersonnii* (Lacépède), in the lens capsule of which they develop into diplostomula. When ingested by a Herring Gull these larvae attain sexual maturity in the bird's intestine. The conclusion of the first mentioned authors was based on three lines of evidence: first, the metacercariae occurred only in snails infected with the cercaria of *D. flexicaudum;* second, the metacercariae agreed in structure with the description of the metacercaria of *D. flexicaudum* as given by Hughes and Berkhout (1929, Pap. Mich. Acad. Sc., Arts and Letters **10**: 483–488) ; and third, they had observed all the developmental stages from cercaria to metacercaria in the numerous snails which they had autopsied. However, these authors did not test whether these precociously developed meta-

cercariae were infective and able to develop into adults if fed to a suitable final host.

Since precocious metacercariae of *D. flexicaudum* were appearing as usual in routine autopsies of *S. emarginata angulata* collected in the Douglas Lake Region during the summer of 1939 it seemed desirable to test the infectivity of these larvae at this time. On August 11 about 150 diplostomula taken from one specimen of *S. emarginata angulata* were fed by dropper to a six weeks old chicken. Five days later twelve diplostomes were recovered from the small intestine of the bird. The small number of worms is taken to mean that the chicken is not a satisfactory host for *D. flexicaudum* although it is possible to conclude that the metacercariae used were not all sufficiently developed to be infective. Though small, the worms were almost mature and one of them contained two eggs. Measurements of five whole mounts are given to indicate the size and proportions of the worms which were fixed without pressure in warm Gilson's solution. Total length 1.33–1.50 mm, mean 1.37 mm. Length of forebody 0.82–0.90 mm, mean 0.85 mm. Width of forebody 0.41–0.53 mm, mean 0.48 mm. Width of hindbody 0.28–0.34 mm, mean 0.31 mm. Diameter of oral sucker 0.077–0.086 mm, mean 0.083 mm. Diameter of pharynx 0.054–0.058 mm, mean 0.056 mm. Transverse diameter of acetabulum 0.086–0.101 mm, mean 0.092 mm.

Study of whole mounts and sections of the worms revealed no significant differences except size between them and the description of the adults of *D. flexicaudum* as given by Van Haitsma. It thus appears that precocious metacercariae of this species which have developed in the first intermediate host are not only normal in appearance but are infective. Consequently, the life cycle of this species may be modified, experimentally at least, to omit the second intermediate host which is a fish.—Louis Olivier, *New York University, University Heights, New York.*

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No. 2, April 10
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No. 4, August 9
No. 5, October 10
No. 6, with December Supplement, December 8